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RATE OF PREGNANEDIOL EXCRETION FOLLOWING INTRAPORTAL OR INTRASYSTEMIC INTRAVENOUS ADMINISTRATION OF PROGESTERONE IN HUMAN SUBJECTS*

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INTRODUCTION

THE inactivation of progesterone and its conversion to pregnane-3α-20α-diol by the liver, is by now a well documented fact. In the studies of Masson and Hoffman (1) and Ferin and Merteus (2), progesterone was found to be much less effective in inducing progestational proliferation in the rabbit uterus after oral than after parenteral administration; but its oral effectiveness increased ten-fold after partial hepatectomy (1). Similar findings were obtained earlier by Kochakian, Haskins and Bruce (3). (Engel (4), however, observed practically no difference, except with very small doses, in the effectiveness of progesterone in this reaction when the intraportal was compared with the systemic route of administration.) Dosne (5) similarly found progesterone less effective in preventing fibrotic changes in guinea-pig uteri after implantation of progesterone pellets in the spleen than after subcutaneous implantation. Selye and Stone (6) showed that the anesthetic effect of this hormone was decreased when it

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was given by mouth and that hepatectomy almost completely restored its anesthetic effectiveness.

The experiments of Masson and Hoffman (1) and of Selye (6) demonstrate that the liver, rather than the gut, is the site of inactivation. Several other investigators have shown the same thing in a different way. Thus, regardless of whether progesterone be given by mouth or parenterally, the *amount* of pregnanediol formed from a given amount of progesterone is always about the same (7–9). Pregnanediol has also been found in the bile after administration of progesterone, but (in contrast to the steroid estrogens, which seem to be excreted into the bile) progesterone itself does not appear in the bile (10). The earlier conflicting reports on the ability of the liver to metabolize progesterone and in particular to convert it to pregnanediol seem to have been resolved by the work of Taylor (11), who showed that pregnanediol, as well as 4 other steroid metabolites, was formed by liver tissue *in vitro*. The amount of pregnanediol formed—9.5 per cent of the amount of progesterone originally present—agrees remarkably well with the conversion ratio found by most investigators in *in vivo* studies.

Thus, although it can be accepted as a fact that the liver inactivates progesterone and converts it to pregnanediol, the possibility that there may be other sites of metabolism has not been eliminated. We have set up and carried out an experiment designed to compare the *rate* of excretion of pregnanediol after injection of progesterone into a systemic vein with its rate of excretion after portal intravenous administration, in the hope that this might throw some further light on this problem.

METHODS AND MATERIALS

Ten human female subjects were studied. Nine had been admitted to the Gynecology Service of University Hospital for pelvic surgery and 1 was a private patient with essential dysmenorrhea who was studied as an outpatient. Urine was collected from each subject over a 12-hour to 24-hour period prior to the time of injection of progesterone. At laparotomy, an omental vein was isolated in 6 patients and an injection of progesterone made directly into it; in 3 patients such an injection was made into a systemic vein (the antecubital) at an equivalent time during surgery. The outpatient received an injection of progesterone into the antecubital vein on each of two occasions about a month apart. In her case the dose each time was 50 mg.; in the table and graphs, the values of pregnanediol for this patent are the means of the two runs. The amount of progesterone given to the patients who were operated on was between 82 and 100 mg. The hormone was administered in a solution of propylene glycol and human serum albumin according to a method of preparation described previously by one of us (12).

An indwelling catheter was inserted into the bladder of each of the 9 surgical patients, and *immediately* on finishing the injection of progesterone (which occupied a period of about ten minutes) a fresh collecting bottle was placed under the drainage tube from the bladder and the urine collection was started. No attempt was made to invoke diuresis. With the few exceptions noted in the graphs, total collections of urine were made for

the periods at the end of 2, 4, 6, 8, 12, 16, 24 and 48 hours after the progesterone injection in 8 subjects and similarly up to 24 hours in 1 subject. The outpatient, for each of her two runs, collected her own urine in total batches at the end of 2, 4, 6, 8, 12 and 24 hours. The patients' ages ranged from 24 to 48 years. Five of the patients who were operated on had panhysterectomies; 1 had a bilateral partial salpingectomy, 1 a bilateral partial salpingectomy and uteropexy, 1 a right ovariectomy and 1 a myomectomy and appendectomy. A dilatation and curettage was performed on all but 1 patient, and the curettings were examined microscopically. Secretory endometrium was found in 4, and proliferative endometrium in the remaining 4. The 1 patient who had no curettage prior to operation had been having regular cycles. She underwent operation on the sixth day of her cycle, and so was presumed to be in the proliferative phase.

Pregnanediol determinations were made in duplicate by the method of Sommerville, Gough and Marrian (13).

RESULTS

Table 1 is a summary of the vital data, procedures, and the percentile conversion of progesterone to pregnanediol for each patient. The patterns of excretion of pregnanediol are shown in Figure 1 as a scattergraph of the individual excretions for each period, regardless of the patient, but the two

TABLE 1. CLINICAL DATA AND EXPERIMENTAL CONDITIONS OF STUDY OF PREGNANEDIOL EXCRETION AFTER INTRAPORTAL OR INTRASYSTEMIC ADMINISTRATION OF PROGESTERONE, SHOWING THE PERCENTAGE OF ADMINISTERED PROGESTERONE APPEARING AS URINARY PREGNANEDIOL

Patient, Age & Color*	Surgical procedure	Endometrial curettings	Time from LNMP† to injection	Progesterone		Total urinary pregnanediol (as % of progesterone dose)
				Dose (mg.)	Site of intravenous injection	
CK, 24 N	Myomectomy and appendectomy	None	6 days	90	Omental	5.9
FB, 32 W	BPS‡ & uteropexy	Proliferative	15 days	100	Omental	6.2
HH, 37 W	Hysterectomy	Proliferative	4 days	82	Omental	10.3
AT, 38 W	Hysterectomy; USO§	Secretory	19 days	95	Omental	7.6
DS, 32 W	Hysterectomy; USO§	Secretory (early)	27 days	94	Omental	7.2
PS, 32 N	Hysterectomy; BSO‖	Secretory (early)	59 days	95	Omental	6.2
						Mean 7.2
FS, 39 W	Hysterectomy; BSO‖	Proliferative	13 days	100	Antecubital	10.6
FC, 25 W	BPS‡	Proliferative	76¶ days	94	Antecubital	11.2
TH, 34 W	Rt. oophorectomy	Secretory	58 days	100	Antecubital	3.4
LT, 27 W	None (outpatient)	None	Day 14 of cycle	50	Antecubital	10.1
			Day 9 of cycle	50	Antecubital	8.2 (9.2)
						Mean 8.6

* N = Negro. W = White.
† Last normal menstrual period.
‡ Bilateral partial salpingectomy.
§ Unilateral salpingectomy and ovariectomy.
‖ Bilateral salpingectomy and ovariectomy.
¶ This patient had a slight blood-tinged discharge almost daily since her last period.

groups are compared by showing the means of these excretions for each point in the collection period. The values are shown in Figure 1 as the cumulative percentage of the administered progesterone. In Figure 2 are shown the amounts of pregnanediol excreted per hour, but in order to make comparisons on an equal basis, these values are expressed as percentages of the administered progesterone.

Two things are evident from these graphs: 1) there was practically no difference between the two groups with respect to either *rate* of pregnanediol excretion or *percentile conversion* of progesterone to pregnanediol, and 2) there was a rapid decrease in the rate of excretion of pregnanediol between the fourth and twelfth hour after injection, and a change to a much slower rate of excretion from this time to the end of the forty-eight hours. The decline in excretion rate during the first twelve hours appears to be a straight line on semi-logarithmic coordinates.

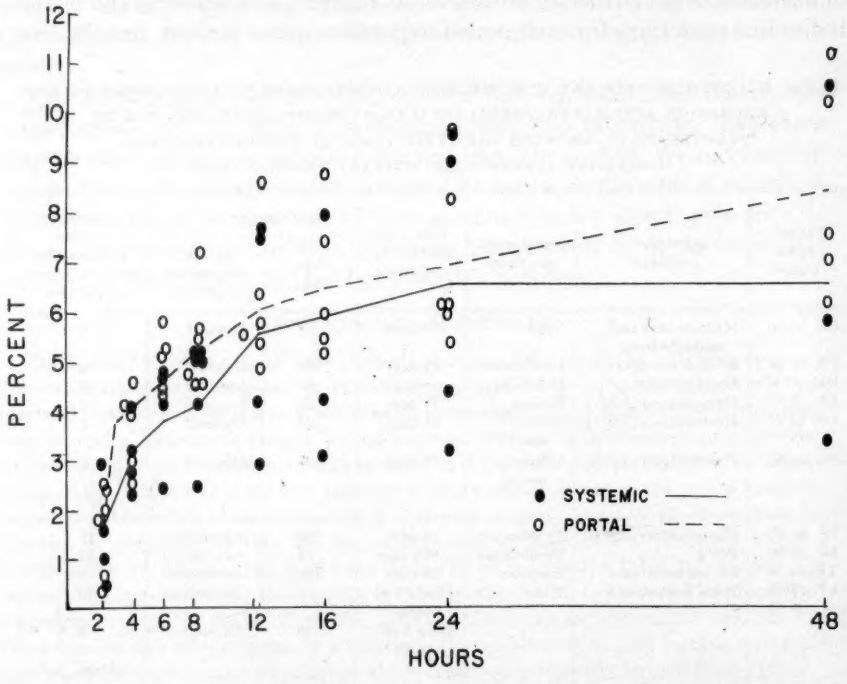

FIG. 1. The percentage of progesterone converted to pregnanediol over a period of forty-eight hours following intraportal or intrasystemic injection of progesterone. (The individual values at each period following injection are shown as a scattergraph, and the mean values for each group are shown by the solid and broken lines as the cumulative percentages.)

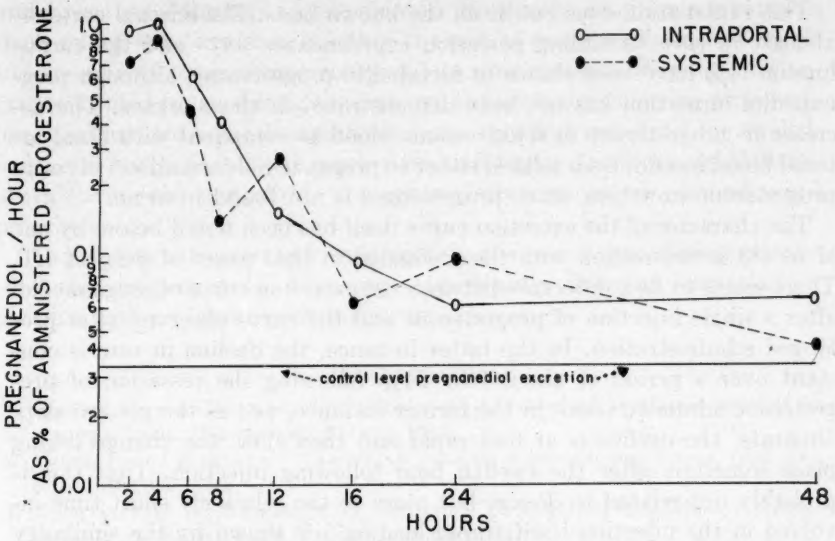

FIG. 2. The decline in pregnanediol excretion following intraportal or intrasystemic injection of progesterone. (Values for pregnanediol are shown as a percentage of the administered progesterone per hour, rather than as milligrams of pregnanediol, in order to compare values in all patients receiving varying amounts of progesterone, on an equal basis.)

DISCUSSION

This study adds one more fact to the accumulating body of information that implicates the liver as the major, if not the only site of inactivation of progesterone and of its conversion to pregnanediol. Our data show that the rate of excretion of pregnanediol after progesterone administration is practically the same after intraportal as after intrasystemic administration. In itself, this finding would not form the basis for this conclusion, but taken in conjunction with the facts that (a) progesterone which has first passed through the liver before being exposed to the rest of the body is devoid of typical progesterone-like biologic activity and (b) the amount of pregnanediol formed from progesterone is the same regardless of the route of administration, it leads to this conclusion as the one that explains most of the available facts in the simplest and most logical way. Progesterone and desoxycorticosterone are the only steroids that are known to give rise to pregnanediol. Since neither progesterone-like, nor desoxycorticosterone-like effects are seen after absorption of progesterone through the portal system, this must mean that along with the over-all inactivation of progesterone by passage through the liver, whatever pregnanediol that is to be formed is formed as part of this total inactivation process.

This explanation does not fit *all* the known facts. The adrenal cortex—at least *in vitro* (including perfusion experiments) (14)—and the corpus luteum (15) have been shown to metabolize progesterone, although pregnanediol formation has not been demonstrated in these organs. The decrease in progesterone in renal venous blood as compared with renal arterial blood has not been related either to pregnanediol formation (16) or to progesterone excretion, since progesterone is not found in urine.

The character of the excretion curve itself has been noted before by one of us (8) in connection with the discussion in that paper of Subject LT. There seems to be a difference between the excretion curve of pregnanediol after a single injection of progesterone and the curve observed after prolonged administration. In the latter instance, the decline in rate is constant over a period of about four days following the cessation of progesterone administration; in the former instance, *i.e.*, as the present data illustrate, the decline is at first rapid and then slow, the change taking place sometime after the twelfth hour following injection. That this is probably not related to dosage but more to the relatively short time involved in the injection itself (rapid loading?) is shown by the similarity between these curves and that for Patient MB in the previous study of pregnanediol excretion after intravenous progesterone (8). The latter patient received 500 mg. of progesterone intravenously each day for ten consecutive days, each amount being given in a period of an hour or less. The curve of pregnanediol excretion, as measured in three eight-hour periods each day, was essentially similar after each injection to those shown here. We have no adequate explanation for this phenomenon.

The late Dr. Henry Guterman reported that the percentage of progesterone converted to pregnanediol was higher (by about 100 per cent) in the luteal than in the follicular phase of the menstrual cycle, as well as higher in viable than in non-viable pregnancies (17). Similar findings have been reported by Venning and Browne (18, 19). We have seen no evidence of this relationship to the cycle in our series; the patients with secretory endometrium at the time of surgery manifested conversion percentages in the same range as those of patients with proliferative endometrium. In a forthcoming study (20) there was also found to be a lack of such relationship between conversion percentage and the viability of a pregnancy.

SUMMARY

The rate of excretion of pregnanediol, and the percentage of progesterone converted to pregnanediol after intravenous administration of progesterone, have been studied in 10 human female subjects.

Of these subjects, 6 received the progesterone through an omental vein,

and 4 received it through a systemic vein. No significant difference between these two groups was seen in either the rate of pregnanediol excretion, or the amount of progesterone converted to pregnanediol. Pregnanediol excretion tended to rise during the first two to four hours following progesterone injection, then to fall rapidly and at a constant rate until the twelfth hour, and finally to fall at a much slower rate up to the forty-eighth hour, at which time the control rate of pregnanediol excretion was reached or approached.

In patients with secretory endometrium the percentage of progesterone converted to pregnanediol was not significantly different from that in patients with proliferative endometrium.

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THE HYPOGLYCEMIC RESPONSE TO INSULIN IN MAN AFTER SULFONYLUREA BY MOUTH*

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IT IS now fairly well established that the sulfonylureas are effective in reducing the hyperglycemia of many patients with diabetes mellitus, especially those in whom the diabetes developed after 40 years of age (1–4). The precise mechanism whereby the sulfonamide derivatives produce a reduction in the blood sugar of animal and man is still unknown. There is general agreement that these compounds do not elicit a hypoglycemic response in the totally depancreatized dog (5) and the severely diabetic alloxanized rat (6). Accordingly, the hypoglycemic response is dependent upon the integrity of the beta cells of the islets of Langerhans and the presence of insulin. Further, the response cannot be due to a diminution in the availability or activity of glucagon, since the alpha cells of the islets of Langerhans are relatively intact in the alloxanized animal. Likewise, the response cannot be due to some nonspecific hepatotoxic effect or a decrease in the activity of the anterior pituitary or adrenal cortex, since such actions would be present in the alloxanized as well as in the normal rat.

The foregoing and other considerations suggest that sulfonylureas may be effective in normal, but not in alloxanized or depancreatized animals, through an increase in the availability of insulin. Such an increase may result from either an increased secretion of insulin into the circulation consequent to direct stimulation of the beta cells of the islets, or to a decreased destruction of insulin by the liver and other tissues consequent to an inhibition of the enzyme which catalyzes the hydrolysis of insulin (insulinase). That the release of insulin into the circulation may play a role in the hypoglycemic response to the sulfonylurea is suggested by the observation that the immediate blood sugar response of healthy subjects is similar to that resulting from the intravenous injection of insulin (7). This immediate response is absent in patients with diabetes mellitus—in whom nevertheless hypoglycemia may develop within several hours after administration of sulfonylurea (3). That the inhibition of insulinase may play a role in the

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† Aided by The Spector Memorial Fund.

persistence of the hypoglycemic response of normal and diabetic subjects to the ingestion of the sulfonylureas is suggested by the fact that the sulfonylureas are non-competitive inhibitors of insulinase *in vitro* and *in vivo* (8). Thus, both mechanisms may be involved in the relatively prolonged hypoglycemia observed in healthy subjects.

If inhibition of insulinase plays a role in the hypoglycemic response of man, then the action of a standard dose of insulin should be enhanced after the administration of sulfonylurea. Accordingly, the hypoglycemic response to exogenous insulin was studied in patients with diabetes mellitus who manifested no blood sugar response to the administration of the sulfonylurea alone.

METHOD

Ten patients with diabetes mellitus were selected for this study, after establishing that the administration of from 50 to 100 mg. of 1-butyl-3-p-tolylsulfonylurea[1] per kilogram of body weight by mouth did not produce a significant reduction of the blood sugar level. In the case of patients who were maintained with long-acting insulins, the medication was changed to regular insulin for three to five days prior to the study. All subjects fasted overnight and were not given any insulin on the morning of the test. Venous blood samples were taken before and at half-hour intervals after the intravenous injection of 0.1 unit of insulin per kilogram of body weight. At the end of the second hour, each subject was given from 50 to 100 mg. of tolylsulfonylurea per kilogram of body weight, as a 2 per cent solution in 0.5 per cent sodium bicarbonate adjusted to pH 8. Blood samples were taken at one and two hours after the administration of the tolylsulfonylurea. The insulin tolerance test was then repeated. The glucose concentration of the blood was determined by Nelson's procedure (9). All data are expressed in terms of mean $\pm$SE.

An equal number of similar patients with diabetes served as controls, in that they were given the sodium bicarbonate solution instead of tolylsulfonylurea.

RESULTS

To facilitate comparison of the blood sugar responses to exogenous insulin before and after the administration of either sodium bicarbonate or the sulfonylurea, the blood sugar concentrations after administration were expressed as percentages of the values immediately prior to each injection of the insulin.

Essentially identical hypoglycemic responses occurred after each injection of insulin in the patient given sodium bicarbonate between the injections (Fig. 1). Thus, there was no statistically significant difference in the percentile decrease in the blood sugar level at each interval after the first and second injections of insulin ($P > 0.05$). Likewise, a repeated-measures analysis of variance (10) revealed no difference between the two hypoglycemic responses ($P > 0.05$).

[1] We are indebted to Dr. C. J. O'Donovan of The Upjohn Company for generous supplies of 1-butyl-3-p-tolylsulfonylurea (Orinase).

That the administration of tolylsulfonylurea alone did not influence the blood sugar level of the patients selected for this study is demonstrated by the dotted curve in Figure 2, in which the blood sugar concentration is expressed in terms of the concentration immediately before the administration of sulfonylurea (388 ± 42 mg. per 100 ml.). The blood sugar levels immediately prior to the first and second injections of insulin were 389 ± 31 and 301 ± 31 mg. per 100 ml., respectively. As anticipated from the results

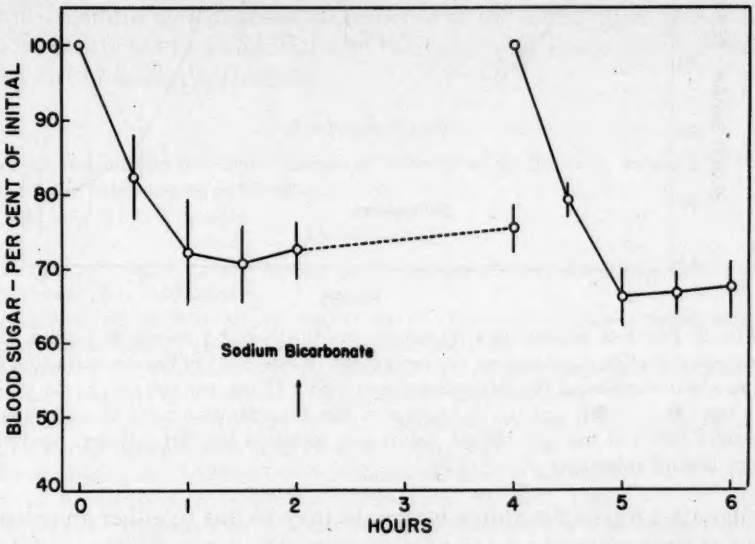

FIG. 1. Effect of sodium bicarbonate on the blood sugar response to insulin. Blood sugar concentration expressed as a percentage of the concentration prior to the injection of insulin at 0 and 4 hours.

of previous tests on these patients, the sulfonylurea did not induce a decrease in the concentration of blood sugar. However, its administration resulted in a more marked response to the same quantity of insulin. The difference between the values obtained at all intervals after the second injection of the insulin was statistically highly significant ($P < 0.001$). A repeated-measures analysis of variance (10) likewise revealed that the difference between the two insulin tolerance curves was statistically highly significant ($P < 0.001$).

DISCUSSION

The administration of consecutive doses of insulin to a patient with diabetes mellitus does not enhance the hypoglycemic response to insulin (Fig. 1). Consequently, the increase in the hypoglycemic response to exogenous

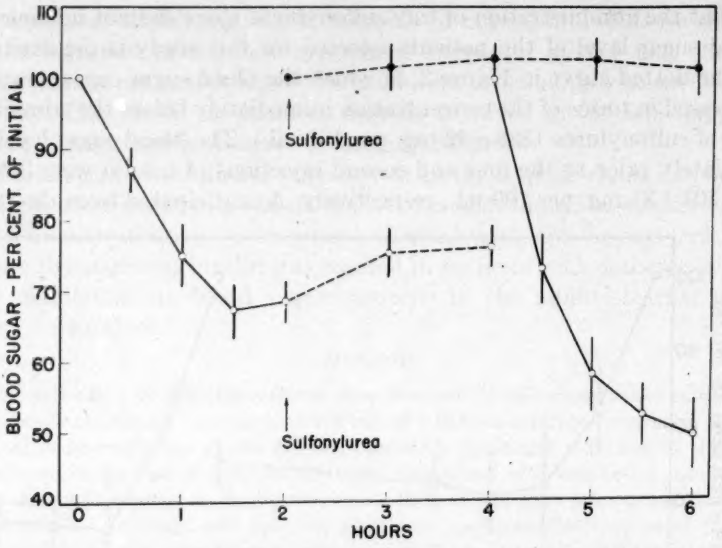

FIG. 2. Effect of sulfonylurea by mouth on blood sugar response to insulin. Blood sugar concentration expressed as: (a) percentage (mean ±SE) of the concentration prior to the administration of the tolylsulfonylurea (388 ±42 mg. per 100 ml.) in the preliminary test (● - - - ●); and (b) percentage of the concentration prior to each injection of insulin (389 ±31 mg. per 100 ml. before first injection and 301 ±31 mg. per 100 ml. before second injection) (○ —— ○).

insulin after tolylsulfonylurea by mouth may be due to either an enhancement of the specific physiologic action of insulin or to a greater availability for such action of the quantity of insulin injected. No evidence is available in support of the possibility that the physiologic action of insulin is increased in the presence of the sulfonylureas (11). On the other hand, a greater availability of insulin may result if the rate of insulin destruction is reduced.

Insulin is destroyed by man and by animals presumably as a result of the action of insulinase (12). Accordingly, it is probable that the hypoglycemic response to the injection of a specific quantity of insulin is due to the physiologic action of only a portion of that quantity. With a decrease in the rate of insulin destruction, a greater proportion of the injected insulin would become available for physiologic action and thereby produce a greater hypoglycemic response. Thus, the administration of a liver extract rich in an insulinase-inhibitor results not only in a decrease in the destruction of insulin by the intact animal (13) but in an enhancement of the hypoglycemic effect of a specific quantity of exogenous insulin (14). Since the sulfonylureas have been shown to exert an insulinase-inhibitory activity concomitant with the hypoglycemic response of normal rats (7),

it is probable that the enhancement of the hypoglycemic response to exogenous insulin described here is due to an inhibition of insulinase and a consequent increase in the proportion of the injected insulin available for physiologic action.

SUMMARY

The administration of 1-butyl-3-p-tolylsulfonylurea by mouth results in an increased hypoglycemic response to exogenous insulin in patients with diabetes mellitus who manifest no response to the sulfonylurea alone. This effect is attributed to an inhibition of insulinase and a consequent decrease in the rate of insulin destruction.

Acknowledgment

We are indebted to Dr. Henry Dolger of New York and Dr. L. L. Pennock of Pittsburgh for the patients used in this study.

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EXCRETION OF EPINEPHRINE AND NOREPINEPHRINE IN VARIOUS EMOTIONAL STATES*

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RESPONSES of the adrenal gland to stress have been the subject of much research (1). Unlike the adrenal cortex, the adrenal medulla as a part of the sympathetic nervous system is intimately connected with the expression of the emotions (2). A study was undertaken to determine the excretion of epinephrine (E) and norepinephrine (NE) of normal and psychiatric patients in various emotional states. Data were obtained on: (a) subjects in both sleeping and waking states, (b) professional hockey players and their coach before and after the game, (c) amateur boxers before and after the contest, (d) neuropsychiatric patients appearing at staff conference, (e) normal subjects in anticipatory states, (f) psychiatric patients receiving the psychotomimetic agent, lysergic acid diethylamide (LSD), and (g) psychiatric patients on whom mental status and Malamud-Sands rating scale records were obtained during the collection period.

The excretion rates of E and NE were studied after graded doses of E and NE were infused. These data were used to estimate the secretion of the amines in the various stress conditions studied.

METHODS

After acid hydrolysis (3), the urine was extracted by the alumina adsorption method of von Euler and Hellner (4) and bioassayed by a modification of the method described by Gaddum and Lembeck (5). The bioassay is based on the quantitative inhibition by the catechol amines of the contraction induced with acetylcholine in an *in vitro* preparation of rat colon and rat uterus. E and NE are approximately of equal potency in their effect on the rat colon, whereas E is some one hundred times as potent as NE in its effect on the rat uterus (6, 7).

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RESULTS

Infusion experiments

Figure 1 depicts the data of 10 E infusions and 9 NE infusions in doses of 0.05, 0.10, and 0.20 micrograms per kilogram per minute (μg./Kg./min.). Pre-infusion urine was collected from 9:00 A.M. to 10:00 A.M., at which time the infusion was started and continued for thirty minutes. The infusion collection represented the period ranging from 10:00 A.M. to approximately 11:00 A.M. Figure 1 shows the hourly excretion for each dosage rate above its control level. In the case of E, only 0.5–1.0 per cent of the total dose infused appeared in the urine, but in the case of NE from 3.0–5.0 per cent of the total dose appeared in the urine in excess of the control level (8). For the NE infusions the relationship of excretion-above-control and dosage seemed to be essentially linear. However, in the case of E infusion, there was a sharp change in the slope at doses above 0.10 μg./Kg./min. Although there was a two-fold increase in the excretion rate with the doubling of the dose from 0.05 to 0.10 μg./Kg./min., there was a seven-fold increase in the excretion rate on subsequent doubling from 0.10 to 0.20 μg./Kg./min. These data served in estimating the approximate secretion rates of E and NE in the studies reported.

Diurnal variation

Six normal subjects consisting of physicians and laboratory personnel who were conducting their usual daily activities, were studied. Each col-

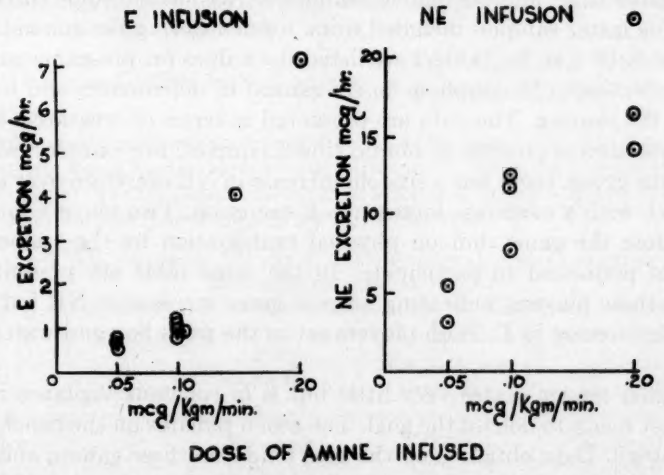

FIG. 1. The excretion of epinephrine (E) and norepinephrine (NE) above control levels after thirty-minute infusion of E and NE in doses of 0.05, 0.10 and 0.20 μg./Kg./min. (*see* text).

TABLE 1. DIURNAL VARIATION OF NE AND E EXCRETION IN 6 NORMAL SUBJECTS*

	NE (μg./hr.)	E (μg./hr.)
Sleep	1.2 ±0.14	0.02 ±0.01
Morning	2.3 ±0.49	0.10 ±0.02
Day	2.4 ±0.60	0.21 ±0.07

* *See* text for times of collection.

lected a sample representing the period of sleeping, a second sample from the time of waking to about 10:00 A.M., and a third sample from 10:00 A.M. to the time of retiring at night. The results are listed in Table 1. NE excretion increased two-fold, and E excretion by five to ten-fold on waking.

Professional hockey

This sport entails considerable aggressive activity in attack and defense. The game is fast and involves marked physical activity and man-to-man contact. Pre-game samples of urine were collected at ten to thirty minutes before game time, and post-game samples were collected some three hours later. The latter samples included urine formed during the contest. Game time was 8:00 P.M. In Table 2 are listed the values for pre-game and post-game collections (20 samplings in six games) of defensemen and forwards who do the skating. The data are presented in terms of creatinine because it was not always possible to obtain timed samples. For samples taken following the game, there was a six-fold increase in NE excretion over the control level, with a moderate increase in E excretion. Two players were sampled before the game, but on physical examination by the trainer, they were not permitted to participate. In the same table are presented the data of these players, indicating no post-game increase in NE but an appreciable increase in E. Both players sat in the press box and watched the game.

The goal tender skates very little but is in constant vigilance in front of the net ready to defend the goal. The coach remains on the bench directing strategy. Data obtained on the goal tender in three games, and on the coach in six games are presented in Table 3. There was a marked increase in both NE and E excretion in the goal tender, but on the average there were no significant changes in the excretion of either amine in the coach.

TABLE 2. EXCRETION OF NE AND E IN MEMBERS OF A PROFESSIONAL HOCKEY TEAM—DEFENSEMEN AND FORWARDS VERSUS NON-PARTICIPATING PLAYERS

Urine collection	Number of hockey players sampled	NE (μg./100 mg. creatinine)	E (μg./100 mg. creatinine)
*Active hockey players (defensemen and forwards)**			
Pre-game	20	2.7±0.43	0.36±0.07
Post-game	20	15.3±2.20	0.95±0.21
		t=5.66	t=2.68
		P=less than .001	P=less than 0.05, greater than 0.01
Two players who did not participate in the game†			
Pre-game	#18	2.2	0.23
Post-game		3.3	0.75
Pre-game	#10	5.6	0.78
Post-game		5.3	1.42

* The approximate hourly excretion is 10% less than the figure given in terms of 100 mg. creatinine when corrected by creatinine coefficient.[1]

† Due to their physical condition.

[1] Data in terms of μg./100 mg. of creatinine may be computed in terms of μg./hr. if the weight of the subject is known. Since the weight of the athletes is highly correlated with muscle mass, and creatinine excretion is dependent on muscle mass, the computation of the formula for creatinine coefficient would furnish the creatinine excretion per unit time for a particular weight (24).

$$\text{Creatinine Coefficient (18–32 for males)} = \frac{\text{Mg. creatinine per 24 hrs.}}{\text{Body wt. in Kg.}}$$

For example, using a creatinine coefficient of 26, it was calculated that the subject weighing 200 pounds would excrete approximately 100 mg./hr. Since the average weight of the hockey players was 180 pounds, the estimate in terms of μg./hr. would be 10 per cent less than that presented in terms of μg./100 mg. of creatinine.

TABLE 3. EXCRETION OF NE AND E IN PROFESSIONAL HOCKEY PLAYERS—GOAL TENDER AND COACH

	Number of samples	Urine collection	NE (μg./100 mg. creatinine)	E (μg./100 mg. creatinine)
Goal tender	3	Pre-game	3.3±0.7	0.45±0.20
		Post-game	9.2±1.8	1.30±0.81
Coach	6	Pre-game	1.8±0.5	0.38±0.09
		Post-game	3.7±0.9	0.43±0.21

Amateur boxing

Six amateur boxers were sampled in the finals of a national tournament. Each boxer was engaged for three rounds, or a total of fifteen minutes from the beginning to the end of the contest. The results in this case were also expressed in terms of creatinine because of the difficulty of obtaining timed urine samples. In Table 4 are presented the results for each boxer studied.

TABLE 4. EXCRETION* OF NE AND E IN AMATEUR BOXERS

Boxer	Urine collection	NE (μg./100 mg. creatinine)	E (μg./100 mg. creatinine)
Ch	Pre-contest	17.9	1.64
	Post-contest	—	—
Sm	Pre-contest	38.1	1.78
	Post-contest	32.4	0.67
Br	Pre-contest	4.2	0.40
	Post-contest	7.0	0.87
Pe	Pre-contest	6.7	0.22
	Post-contest	2.8	0.41
Why	Pre-contest	1.7	1.67
	Post-contest	—	—
Bra	Pre-contest	—	—
	Post-contest	15.9	1.70

* The approximate hourly excretion is 25 per cent less than the value given in terms of 100 mg. of creatinine when corrected by the creatinine coefficient.

Unlike the pre-game E values for the hockey players, the pre-contest E values for the boxers were elevated, with or without further elevation after the contests. The NE excretion was varied; elevated values were observed both before and after the contest. The pre-contest E values in this study were the highest obtained in the so-called "control" samples of all our studies.

Neuropsychiatric patients during staff interview

The conditions of the interview were in marked contrast to those encountered by the athletes. The patient sits across from the psychiatrist doing the interviewing in the presence of some 20 members of the hospital medical staff. All participants are seated and the exchange in this case is

strictly verbal. The setting is serious and to the patient it is very important, because on the basis of his performance decisions are made by the staff which affect his immediate future. Eleven subjects were studied, on 8 of whom control samples were obtained. The interviews took place on Thursday mornings and the control sample was obtained at the same time

TABLE 5. EXCRETION OF NE AND E DURING STAFF CONFERENCE INTERVIEW OF 11 NEUROPSYCHIATRIC PATIENTS

	Number of samples	NE (μg./hr.)	E (μg./hr.)
Interview day	11	2.6±0.4	0.50±0.14
Control day	8	2.3±0.2	0.27±0.12
			P=less than .001

on the next day—Friday. The results are listed in Table 5. There were no changes in NE excretion when the interview day was compared with the control. However, in every subject on whom a control was obtained there was an elevated excretion of E during the interview. As might be expected, there were marked individual variations in this increase. The subjects in general were self-effacing and on their best behavior with no aggressive or active emotional display.

Normal subjects in anticipatory states

Urine collections were made on 11 normal subjects before they underwent the usual insulin tolerance test and the methacholine (Mecholyl) test described by Funkenstein *et al.* (9). The subjects consisted of laboratory personnel and students. There was a varying degree of knowledge and understanding as to the nature of these tests among the group. In Table 6, the variability of the excretion rates may be seen. The subjects who were familiar with the nature of the tests and the hospital setting were quiet and waited patiently; they had normal excretion rates of E and NE. On the other hand, the students and personnel unfamiliar with the tests and the setting were restless, asked many questions of the attending nurse and physician, and were quite anxious about the experiments. Subjects Gi and Dm had very high excretions of NE with normal values for E, and Subjects Ms and Sm had high values for both amines in each instance.

Administration of lysergic acid diethylamide (LSD)

Fourteen psychiatric subjects were given from 150 to 300 micrograms of LSD orally. Seven were chronic schizophrenics, 4 were manic-depressive

TABLE 6. PRE-TEST NE AND E EXCRETION OF 11 NORMAL SUBJECTS*

Normal subjects	Before insulin test		Before methacholine test	
	NE (μg./hr.)	E (μg./hr.)	NE (μg./hr.)	E (μg./hr.)
Ba	3.4	0.35	1.7	0.13
El	3.2	0.16	4.9	0.17
El	4.1	0.17	—	—
Fo	0.9	0.02	—	—
Ma	1.2	0.10	2.8	0.18
Sp	1.5	0.05	1.0	0.20
Zi	1.9	0.15	3.2	0.51
Gi	3.7	0.22	7.7	0.17
Dm	—	—	8.9	0.07
Ma	4.2	0.52	3.2	0.80
Sm	9.0	1.82	3.5	0.96
Pa	—	—	3.2	0.07

* Adapted from data of Elmadjian *et al.* (19).

patients and 3 had involutional depression. The control collection was made from 10:00 A.M. to 2:00 P.M., at which time the LSD was administered. The LSD collection was made from 2:00 P.M. to 10:00 P.M. In this series, the chronic schizophrenics showed no reaction to the LSD in mood, affect or perception. As seen in Table 7A there were no significant changes

TABLE 7. EFFECT OF ADMINISTRATION OF LSD ON NE AND E EXCRETION IN 14 NEUROPSYCHIATRIC SUBJECTS

	Number of samples	NE (μg./hr.)	E (μg./hr.)
A. *Non-reactors*			
Chronic schizophrenia:			
Control	7	2.3±0.4	0.14±0.02
LSD	7	2.0±0.8	0.20±0.03
B. *Reactors*			
Manic depressive state:			
Control	4	2.8±0.5	0.21±0.09
LSD	4	16.7±3.7	1.54±0.58
Involutional depression:			
Control	3	5.6±0.2	0.21±0.05
LSD	3	3.2±1.5	1.24±0.78

in either E or NE excretion. On the other hand, in the manic-depressive patients and those with involutional depression there was a marked increase in E excretion (Table 7B). In the manic-depressive group there was also an increase in NE excretion, but in the involutional depression group there was no change in NE values. In contrast to the schizophrenics, the other two groups manifested marked psychiatric changes following administration of LSD.

Psychotic subjects on whom Malamud-Sands rating scale records were obtained

In 10 psychiatric patients a study was made of the relationship between results of Malamud-Sands rating scale tests and the excretions of E and NE. This scale (10) rates the patient in 22 categories and the data are recorded on a check sheet. On the rating scale each item scored appears on the left-hand side. A designated base line appears through the center of the rating scale. Going from the base line to either the left or the right, the deviations are observed (Table 8). In the preliminary analysis of the data in the 22 categories rated, motor activity and hostility reactions were abstracted and a composite score including these two items was computed. In Figure 2 is shown the relationship of this score to NE excretion. Patients with active aggressive emotional display had higher NE excretions; those with passive self-effacing emotional display had normal levels of NE. In Figure 2, when a line appears through the circles, it indicates that these subjects also had a high excretion of E. The subject indicated by an asterisk had normal NE excretion, but the excretion of E was 2.75 μg. per hour, which is extremely high. This subject showed periodic bursts of excitement with expressions of fear and guilt.

TABLE 8. PORTION OF THE PSYCHIATRIC RATING SCALE OF MALAMUD AND SANDS (10)

FUNCTION	6	5	4	3	2	1	BASE	LINE	1	2	3	4	5	6
APPEARANCE	Bizarre		Decorative		Over-meticulous		NEAT	CARELESS	Slovenly		Untidy	Incontinent		Smearing
MOTOR ACTIVITY	Excited		Agitated		Restless		ACTIVE	QUIET	Under-active		Retarded	Stuporous		
MIMETIC EXPRESSION	Incongruous		Dramatising		Exaggerated		ANIMATED	RESTRAINED	Stiff		Waxy-flexibility	Mask-like		
RESPONSIVITY	Anaklitic		Suggestible		Dependent		FLEXIBLE	RIGID	Stubborn		Resistive	Negativistic		
HOSTILITY REACTIONS	Destructive		Combative		Belligerent		AGGRESSIVE	SELF-EFFACING	Self-deprecating		Self-mutilating	Suicidal		

The ratings obtained for the functions, "motor activity" and "hostility reactions," were added in computing the total scale. When a subject was rated as being agitated and combative the total score would then be 8 to the left of the base line. On the other hand, a subject rated as self-deprecating and retarded would have a score of 5 to the right of the base line.

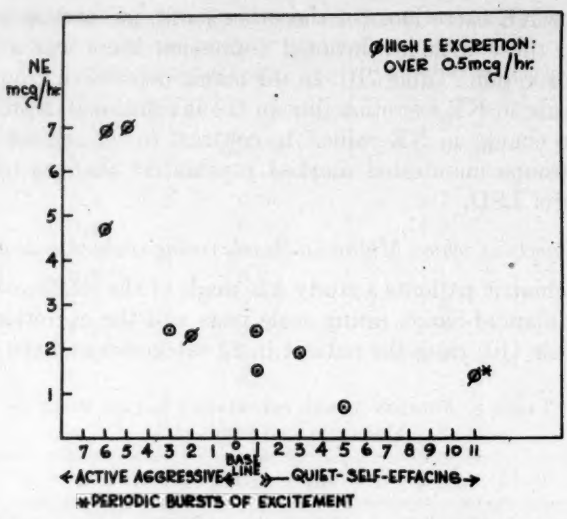

FIG. 2. The relation of norepinephrine excretion to emotional state of neuropsychiatric patients. (The abscissa depicts the composite score for the functions of motor activity and hostility reactions from the Malamud-Sands rating scale, and the ordinate represents the excretion of norepinephrine in μg. per hour during the observation period.)

DISCUSSION

In our studies, three general excretion patterns were evident during emotional states: 1) elevated NE excretion with normal E values, 2) elevated E excretion with normal NE values, and 3) elevated excretions of both NE and E. The diurnal variation data presented in Table 1 may be considered as normal excretion rates with which to compare excretion rates during various stresses.

In anticipatory states (*e.g.*, Subjects Dm and Gi, Table 6) and in hockey players during a moderately tense game, we observed high NE excretion with slight elevated E excretion. In the involutional-depressive patients receiving the psychotomimetic agent, LSD, and in the neuropsychiatric patients during an interview, there was elevated excretion of E with little elevation in that of NE. Finally, when an intense emotional display was evident, such as in manic-depressive patients treated with LSD, hockey players, amateur boxers, and disturbed neuropsychiatric patients, both NE and E excretions were elevated. When increased excretion of NE was present, there was ample evidence of aggressive, active emotional display. In cases of tense, anxious but passive display, the excretion of E was frequently elevated.

In studies of airmen, von Euler reported that during the air-borne period there was increased excretion of E, regardless of whether ground personnel

or pilots were sampled (11). This result is similar to the excretion patterns obtained when subjects operated the Werthessen-Hoagland pursuitmeter which simulates the operation of controls of a plane (8). As might be expected, the degree of increase was much smaller in the latter study. Further reports from von Euler's laboratories indicate that increased NE and E excretions are found in athletes who participate in skiing and running events (12). In surgical shock, there is high NE excretion with or without elevated E excretion (13).

The question arose during our studies whether NE excretion could be due to motor activity per se. This is highly unlikely, since several subjects with marked emotional disturbances who were lying down or sitting had high NE excretion with or without elevated E excretion (*e.g.*, Gi, Dm, Sm; Table 6 and Fig. 2). Furthermore, in terms of physical movement (generalized exercise), the goal tender in the hockey study remained in one place during the whole playing time, yet he manifested a marked increase in NE excretion. To test the effect of generalized exercise, experiments were then conducted in which Subject El raked grass for two hours; this work involved considerable walking and movement of the muscles in the shoulders and arms. This subject was not emotionally involved and, at the end, he was physically tired. The excretion of NE was normal (2.2 μg. per hour), but the excretion of E was elevated (0.35 μg. per hour). Two-hour samples were obtained on 2 laborers—1 who worked at mixing mortar and carrying bricks, and 1 who unloaded trucks and was a pick-and-shovel man. The former had an NE excretion of 3.2 μg. per hour and an E excretion of 0.54 μg. per hour, whereas the latter had an NE excretion of 5.7 μg. per hour and an E excretion of 0.12 μg. per hour. It may be concluded that exercise per se (that is, without marked emotional involvement) is not a major factor in determining the high NE values observed, such as those in the boxers or in the hockey players.

The secretion rate levels of the catechol amines E and NE may be estimated by comparing the excretion data obtained during the various stresses with those of the infusion experiments (Fig. 1). Under certain conditions of stress, such as hockey players during the game, the output of NE is of the same order as that during infusion of 0.2 μg. of NE per Kg. per minute, and the output of E is of the same order as that during infusion of from 0.10 to 0.20 μg. of E per Kg. per minute. These data are comparable to those presented by von Euler (14).

Since doubly adrenalectomized humans excrete little or no measurable amounts of E, it seems safe to assume that practically all the E appearing in the urine in normal subjects is secreted from the adrenal medulla (8, 15). NE values for subjects with the adrenals removed are within the normal range. Furthermore, in studies in which the diurnal variation of adre-

nalectomized subjects was determined, there was an increase in NE excretion in the waking state over that in the sleeping state—similar to the increase observed in normal subjects (8). These results indicate that, in all probability, most of the NE excreted is extra-adrenal in origin. Data presented by von Euler on the relative concentrations of E and NE in the adrenal medulla and peripheral postganglionic fibers of the sympathetic nervous system indicate that there is ten times more E than NE in the human adrenal, and that practically all of the neurohormone present in the peripheral nerves is NE. The demonstration of NE secretion following stimulation of postganglionic nerves also adds to the evidence that NE is the peripheral neurohormone of the sympathetic nervous system (16, 17).

Qualitative differences of sympathico-adrenal discharge have been demonstrated by varying the intensity of electrical stimulation of the hypothalamus (18). Evidence also seems to indicate that the central sympathetic representation involved with the secretion of E is different from that involved with the secretion of NE. Hypoglycemia induced by administration of insulin selectively increases the excretion of E without concurrent elevation in the excretion of NE (19, 20). On the other hand, during the hypotension induced by administration of methacholine, although there is no significant increase in E excretion there is a distinct increase in NE excretion when compared with the NE excretion observed followihg an insulin test in the same subject (20). Changes from the recumbent to the standing position result in an increased excretion of NE with no change in E (21). Experimental data of Gellhorn support the view that hypotension induces secretion of NE (22).

With a brief review of experimental data available, one may speculate as to the location of the areas of central sympathetic representation concerned with NE and E excretion. Evidence that electrical stimulation of the posterior hypothalamus induces a sharp increase in blood pressure is well established (23). That this occurs in the absence of the adrenal has also been noted. Hyperglycemia which is induced by stimulation of the lateral hypothalamic region depends on the integrity of the adrenal medulla, since it does not occur when the splanchnic nerve is cut. The inference may be drawn that the posterior hypothalamus is concerned more with secretion of NE, whereas the lateral hypothalamus is concerned more with secretion of E. However, the vasomotor center in the medulla oblongata—the region of the floor of the fourth ventricle—is by far the most sensitive area related to E secretion (2).

SUMMARY

The excretion of epinephrine (E) and norepinephrine (NE) was determined in athletes (hockey players and boxers), normal subjects in anticipa-

tory states, and psychiatric subjects. In the last group, mental status and rating scales were also studied. The results support the hypothesis that active, aggressive emotional displays are related to increased excretion of NE, with or without increased excretion of E, whereas tense, anxious but passive emotional displays are related to increased excretion of E in association with normal excretion of NE.

From infusion studies it was found that, under the foregoing conditions of stress, secretion rates of NE rose to levels comparable to those during infusion of 0.2 μg. of NE per Kg. per minute, whereas the highest E levels were comparable to those during infusion of 0.1 μg. of E per Kg. per minute.

The sources of the NE and E are discussed, as well as the central autonomic representations controlling their secretion.

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EFFECT OF CORTICOTROPIN AND 9α-FLUOROHYDROCORTISONE ON URINARY STEROIDS IN CUSHING'S SYNDROME

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UNTIL comparatively recently, knowledge of steroid excretion in adrenocortical hyperfunction was more valuable in the adrenogenital syndrome than in Cushing's syndrome, owing to the availability of reliable and well-established methods for the estimation of urinary 17-ketosteroids, upon which the diagnosis and management of adrenal virilism are largely based. In Cushing's syndrome, the urinary 17-ketosteroids may be either normal or only slightly elevated. However, with the development of sensitive and relatively simple methods for the estimation of urinary 17-hydroxycorticoids, it has been found that these compounds are more often raised, and to a greater extent, than the 17-ketosteroids.

When the diagnosis of Cushing's syndrome has been made, it is important to determine the nature of the adrenocortical abnormality—whether it be malignant tumor, simple hyperplasia or benign adenoma. Techniques have been tried, similar to those which have been found to be useful in cases of the adrenogenital syndrome. Thus, in the latter condition, Laidlaw *et al.* (1) observed that the administration of corticotropin induced a greatly increased output of 17-ketosteroids in cases due to adrenocortical hyperplasia, but not in cases due to malignant tumor. Similarly, the administration of corticotropin to patients suffering from Cushing's syndrome resulted in a greatly increased excretion of 17-hydroxycorticoids in cases due to hyperplasia, but not in cases due to carcinoma (1).

The use of cortisone in the determination of the causative lesion of adrenal virilism is now well known. Jailer *et al.* (2) showed that in cases due to simple adrenal hyperplasia endogenous corticotropin was inhibited by cortisone, with a resultant fall in urinary 17-ketosteroids, whereas in cases of malignant tumor no depression was observed. Thus, in contradistinction to adrenal hyperplasia, malignant tumors have been regarded as autonomous of pituitary control. It now appears, however, from a recent report by Gallagher *et al.* (3) that this distinction is not invariable. These

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authors have described a case of metastasizing adrenocortical carcinoma which (unlike all previously reported cases) responded to corticotropin by a rise in urinary steroids, and to cortisone by a fall in urinary 17-ketosteroids.

In Cushing's syndrome, the 17-ketosteroids may be an unreliable guide and any effect on the 17-hydroxycorticoid excretion would be effectively masked by the cortisone administered. Recently, a halogenated derivative of hydrocortisone, 9α-fluorohydrocortisone, has been produced. Goldfien *et al.* (4) have shown it to be at least twenty times as active as cortisone for maintenance therapy and to be a very potent inhibitor of corticotropin. These authors found that endogenous corticotropin in a normal person could be inhibited by as little as 1 to 2 mg. daily of fluorohydrocortisone. With dosages of this order, no interference with the estimation of 17-hydroxycorticoid is encountered. Forsham and Thorn (5) originally proposed that fluorohydrocortisone in a dosage of 1 mg. six-hourly for two days could be used as a test to distinguish Cushing's syndrome due to adrenocortical hyperplasia from that due to malignant tumor, since in the former case a marked depression of 17-hydroxycorticoids would occur and in the latter case no response would be obtained. Further experience, however, following reports by Laidlaw *et al.* (1) and Cope and Harrison (6), indicated that in some cases of proved adrenal hyperplasia the expected fall in 17-hydroxycorticoid excretion did not occur even with large doses of fluorohydrocortisone.

The following report is concerned with the effect of fluorohydrocortisone on urinary 17-hydroxycorticoids and 17-ketosteroids in 3 cases of Cushing's syndrome due to adrenocortical hyperplasia. In 2 of these cases the effect of corticotropin was also observed.

METHODS

Urinary 17-hydroxycorticoid excretion was estimated by the method of Reddy (7), based upon the original method of Reddy, Jenkins and Thorn (8), and further modified by Smith *et al.* (9). Normal values ranged from 3.0 to 10.8 mg. per twenty-four hours, using cortisone as a standard.

Urinary 17-ketosteroids were estimated by the method proposed by the Medical Research Council (10).

CASE REPORTS

Case 1

C.L., a 26-year-old single female, was first seen in September 1955 with a history of having gained 35 pounds in weight during the previous eighteen months. Irregular menstruation had begun nine months previously and for the last three months there had been complete amenorrhea. She had noticed increasing hair growth over the face and

back, rounding of the face, and striae over the flanks. Recently she had complained of lumbar backache.

Physical examination (Fig. 1). She was moderately obese, weighing 146 pounds, and the face was rounded and plethoric. Excessive hair and severe acne were present on the face and back. Purplish striae were visible on the iliac crests, buttocks and thighs. Blood pressure was 135/95. The urine was normal.

Laboratory investigations. The blood hemoglobin level was 14.5 grams per 100 ml.,

FIG. 1. *Case 1.*

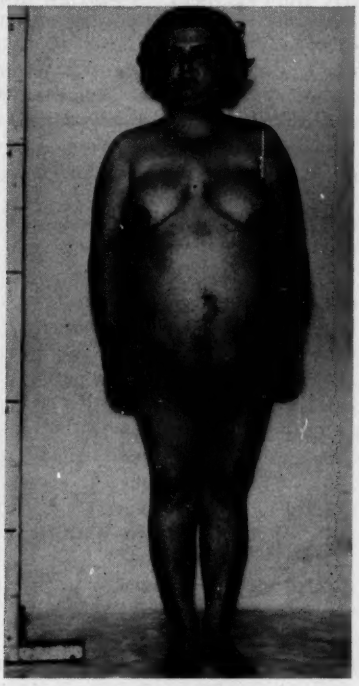

FIG. 2. *Case 2.*

red blood cells 5.6 million per cu. mm., and white blood cells 11,000 per cu. mm. Glucose tolerance was normal. Values for serum sodium, potassium, chlorides and alkali reserve were all normal. Radiologic examination of the pituitary fossa showed no abnormality and there was no osteoporosis of the spine; an intravenous pyelogram and presacral oxygen insufflation revealed normal adrenal outlines.

Progress. In view of the relative mildness of the condition when first seen, no radical surgical treatment was considered. During the next six months, the striae deepened, backache became more severe, and there was further rounding of the face. As a preliminary measure, unilateral adrenalectomy was advised.

Operation. At laparotomy no evidence of tumor was found and a left adrenalectomy was performed.

Pathology. The left adrenal weighed 6 grams; section showed the cortical cells to be well filled with lipoid, but there was no evidence of tumor.

Results of steroid analyses. The relevant data concerning steroid excretion are shown in Table 1. It can be seen that the 17-hydroxycorticoid excretion was moderately elevated (16.0–17.0 mg. per twenty-four hours), whereas 17-ketosteroid excretion was normal. Fluorohydrocortisone, 1 mg. six-hourly for two days, caused an unmistakable fall in both steroids. On

TABLE 1. URINARY STEROID RESPONSE IN CASE 1 TO FLUOROHYDROCORTISONE AND CORTICOTROPIN

Date	Treatment	Urinary 17-OH-corticoids (mg./24 hrs.)	Urinary 17-ketosteroids (mg./24 hrs.)
1956			
Feb. 7	None	16.0	10.5
14	None	17.0	6.3
15	Fluorohydrocortisone, 1 mg. q. 6 hrs.	11.8	8.1
16	Fluorohydrocortisone, 1 mg. q. 6 hrs.	4.2	4.9
17	None	9.5	
18	None	15.6	
20	None	16.3	
23	None	14.3	8.2
24	ACTH, 40 units q. 12 hrs.	19.2	10.0
25	None	50.7	19.6
26	None	19.2	

stopping the drug, the 17-hydroxycorticoids rose to their previous level within two days. The administration of corticotropin (in the form of 40 units twelve-hourly of the zinc phosphate preparation for one day) caused a great increase in 17-hydroxycorticoids to 50.7 mg. and a smaller rise in 17-ketosteroids to 19.6 mg.

Case 2

P.S., a 27-year-old single female, was first seen in February 1955 with a history of having gained 28 pounds in weight during the last year, of increasing hair growth on the chin and abdomen, and oligomenorrhea. Recently she had noticed purple striae on the abdomen and breasts.

Physical examination (Fig. 2). The patient was obese, weighing 170 pounds. Fat was especially prominent over the abdomen and back. The face was rounded and plethoric. Acne was present on the face, neck and back. Excessive hair was visible on the face, back, and areolae of the breasts, and the pubic hair was of the male type. There were

purple striae on the abdomen and breasts. Blood pressure was 170/110. The urine was normal.

Laboratory investigations. The blood hemoglobin level was 16.3 grams per 100 ml., and white blood cells 14,400 per cu. mm. Glucose tolerance was initially normal. Values for serum sodium, potassium, chlorides and alkali reserve were all normal. Radiologic examination of the pituitary fossa and of the spine showed no abnormality. An intravenous pyelogram was normal.

Progress. She was observed for twelve months, during which time there was a slow increase in the signs and she became more dyspneic. A glucose tolerance test, twelve months after the first one, showed a fasting blood sugar level of 119 mg., rising to 214 mg. per 100 ml. after one hour, associated with heavy glycosuria.

Operation. At laparotomy no evidence of adrenal tumor was found. A left adrenalectomy was performed as a preliminary measure.

Pathology. The adrenal gland was enlarged and weighed 10.8 grams; section showed diffuse hyperplasia, and the cells contained abundant lipoid.

Results of steroid analyses. The values for urinary steroid excretion are shown in Table 2. It will be seen that the urinary 17-hydroxycorticoid

TABLE 2. URINARY STEROID RESPONSE IN CASE 2 TO FLUOROHYDROCORTISONE AND CORTICOTROPIN

Date	Treatment	Urinary 17-OH-corticoids (mg./24 hrs.)	Urinary 17-ketosteroids (mg./24 hrs.)
1955			
Dec. 13	None	18.5	16.9
1956			
Mar. 20	None	17.6	17.6
Apr. 21	None	13.8	17.2
23	None	22.2	18.5
24	None	21.2	
25	None	15.6	
26	Fluorohydrocortisone, 1 mg. q. 6 hrs.	16.8	7.2
27	Fluorohydrocortisone, 1 mg. q. 6 hrs.	10.5	9.0
28	Fluorohydrocortisone, 1 mg. q. 6 hrs.	6.9	10.2
29	None	12.9	22.5
30	None	22.3	28.0
May 1	None	20.1	18.9
2	ACTH, 40 units q. 12 hrs.	28.8	18.8
3	None	38.4	16.2
4	None	26.2	15.8
10	None	19.3	18.5
11	ACTH, 40 units q. 12 hrs.		16.9
12	None		15.0

levels were raised and ranged from 13.8 to 22.2 mg. per twenty-four hours. Fluorohydrocortisone, 1 mg. six-hourly, caused a fall to 6.9 mg. on the third day of administration. The effect on urinary 17-ketosteroids was a reduction from 18.5 mg. to 7.2 mg. per twenty-four hours, but this occurred on the first day and was not sustained at this level for the next two days. On stopping the drug, the level of both steroids rose within twenty-four hours, the 17-ketosteroids rising above the previous levels, indicating a rebound effect. The administration of corticotropin-zinc phosphate, 40 units twelve-hourly for one day, resulted in a rise of urinary 17-hydroxycorticoids to 38.4 mg. In contrast, the 17-ketosteroids did not rise.

Case 3

W.H., a 22-year-old married man, was first seen in September 1953 with a history of increasing roundness of the face and excessive growth of hair on the back. There had been no increase in weight. During the previous two months purple striae had appeared over the hips. There was excessive thirst and lumbar backache. Mentally, he had become difficult to manage because of maniacal outbursts.

Physical examination (Fig. 3A). He was mentally disoriented. The face was rounded, plethoric and showed severe acne. The limbs were slender and there was a moderate kyphosis and excessive hair on the back. Purple striae were present over the hips and buttocks. Blood pressure was 170/130. The urine contained no albumen, but there was pronounced glycosuria.

Laboratory investigations. The blood hemoglobin level was 16.0 grams per 100 ml., white blood cells 12,200 per cu. mm., and eosinophils 30 per cu. mm. The concentration of serum sodium was 325 mg., of potassium 8.5 mg., and of chlorides 435 mg. per 100 ml., with an alkali reserve of 112 vols. Radiologic examination of the skull showed no abnormality, but there was evidence of osteoporosis in the thoracic and lumbar vertebrae. An intravenous pyelogram and presacral oxygen insufflation revealed normal adrenal outlines. Urinary 17-ketosteroids were 42 mg. per twenty-four hours, but the value for 17-hydroxycorticoids was not available at this time.

First operation (October 28, 1953). Subtotal adrenalectomy was performed, involving the removal of the whole of the right adrenal and two thirds of the left adrenal.

Pathology. The right and left adrenal glands weighed 11 grams and 6 grams, respectively; section showed normal architecture and the cells contained much lipoid. There was no evidence of a tumor.

Progress. Physically and mentally there was great improvement following subtotal adrenalectomy (Fig. 3B) and he remained well for about eighteen months. By October 1955 the appearance of Cushing's syndrome was again evident, including the purple striae (Fig. 3C). An interesting feature was a deepening brown pigmentation of the face, axillae, groins and buccal mucosa. The blood pressure had risen to 220/110. A glucose tolerance test showed a diabetic blood sugar curve. Radiographs of the spine indicated increased osteoporosis. In an attempt to treat this recurrence of the disease, irradiation of the pituitary was carried out in a dosage of 4000 r over a period of four weeks. Four months later no benefit was apparent, either in the clinical state or in the steroid excretion (Table 3). In March 1956 there was deterioration in vision with hypertensive retinopathy and further surgical intervention was necessary.

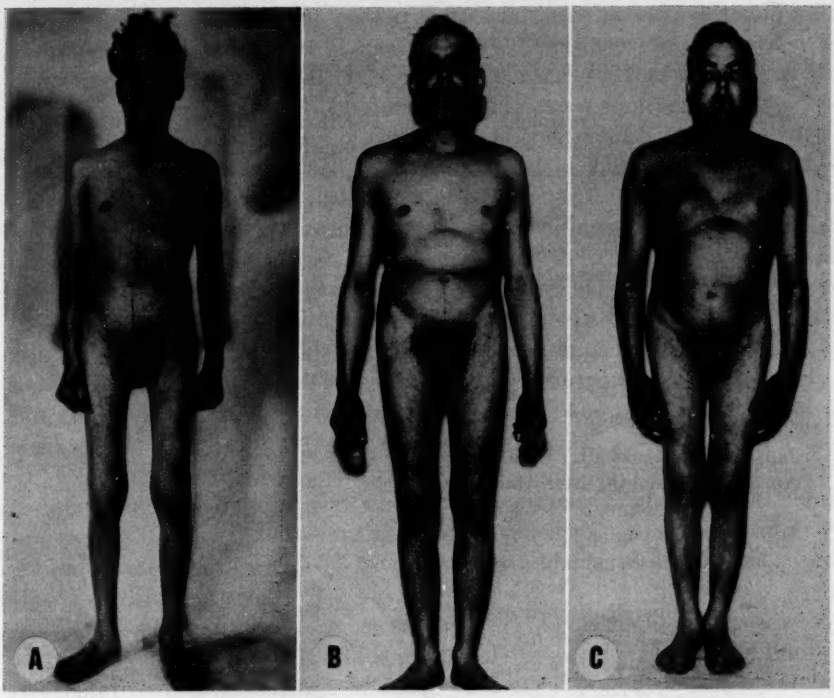

FIG. 3. *Case 3.* **A.** Patient W.H. before first operation (subtotal adrenalectomy). **B.** In a state of remission, four months after the operation. **C.** Relapse, two years after the operation.

Second operation (April 25, 1956). Total adrenalectomy was performed. At operation the left adrenal remnant was seen to be greatly enlarged and was removed.

Pathology. The left adrenal gland weighed 11 grams; section showed a diffuse hyperplasia of cortical cells containing abundant lipoid. There was no evidence of a tumor.

Results of steroid analyses. Urinary steroid estimations are shown in Table 3. It can be seen that the 17-hydroxycorticoid level continued to rise in spite of radiotherapy directed at the pituitary, and this was in keeping with the clinical progression of the disease. Fluorohydrocortisone, 1 mg. six-hourly, resulted in only a slight fall in 17-hydroxycorticoids and 17-ketosteroids, which was considered not sufficient to exclude spontaneous variation. Increasing the dosage to 2.5 mg. six-hourly produced no effect. A corticotropin test was not thought to be justifiable in view of the severity of the hypertension.

Following total adrenalectomy, fluorohydrocortisone (0.5 mg. twice daily) was substituted for cortisone (12.5 mg. twice daily) as maintenance

TABLE 3. URINARY STEROID RESPONSE IN CASE 3 TO PITUITARY IRRADIATION, FLUOROHYDROCORTISONE AND TOTAL ADRENALECTOMY

Date	Treatment	Urinary 17-OH-corticoids (mg./24 hrs.)	Urinary 17-ketosteroids (mg./24 hrs.)
1953			
Oct. 8	None		42.0
28	*Subtotal adrenalectomy*		
Nov. 13	3 wks. after subtotal adrenalectomy		6.0
1955			
Nov. 5	None	19.0	
Dec. 9	During irradiation of pituitary	21.0	18.9
1956			
Jan. 24	7 weeks after irradiation	30.3	21.4
Apr. 3	4 months after irradiation	30.5	18.0
4	Fluorohydrocortisone, 1 mg. q. 6 hrs.	28.4	15.9
5	1 mg. q. 6 hrs.	24.0	14.7
6	None	31.9	17.9
7	Fluorohydrocortisone, 2.5 mg. q. 6 hrs.	28.0	17.0
8	2.5 mg. q. 6 hrs.	29.5	14.1
9	2.5 mg. q. 6 hrs.	26.7	16.8
25	*Total adrenalectomy* Cortisone, 12.5 mg. q. 12 hrs. (maintenance)		
May 12	Fluorohydrocortisone, 0.5 mg. b.i.d. substituted	0	
15	Cortisone, 12.5 mg. q. 12 hrs. resumed	8.4	

therapy, whereupon the 17-hydroxycorticoids fell to zero. This demonstrated the completeness of the surgical procedure. On resumption of cortisone (12.5 mg. twice daily) the excretion rose to 8.4 mg. per twenty-four hours.

DISCUSSION

The 3 patients described were all suffering from Cushing's syndrome which, at operation, was shown to be due to adrenocortical hyperplasia. Important differences, however, were observed in the response of the urinary steroids to fluorohydrocortisone and corticotropin. Case 1 was clinically the least severe and reacted to 1 mg. of fluorohydrocortisone

every six hours by a definite depression of both 17-hydroxycorticoid and 17-ketosteroid excretion on the second day. This response provided evidence for the presence of adrenocortical hyperplasia rather than a tumor. In Case 2 there was also an unmistakable depression of urinary 17-hydroxycorticoids at the end of three days of treatment with fluorohydrocortisone in the same dosage (1 mg., six-hourly). The depression of 17-ketosteroids was less sustained.

On the other hand, the reaction of these patients to the administration of corticotropin was different in the 2 cases. Corticotropin-zinc phosphate (11), a potent preparation giving a sustained adrenal stimulation, was used in a dosage of 40 units twelve-hourly for one day. In Case 1 a tremendous rise in 17-hydroxycorticoids occurred, with a much smaller rise in 17-ketosteroids. In Case 2 the 17-hydroxycorticoids again responded briskly, but the 17-ketosteroids failed to rise even when the test was repeated a week later.

Thus the pattern of response to corticotropin in Cushing's syndrome due to adrenocortical hyperplasia, whereby the urinary 17-hydroxycorticoids are greatly increased, is the antithesis of that which occurs in the adrenogenital syndrome, in which 17-ketosteroids are increased at the expense of the 17-hydroxycorticoids (1).

Case 3 was clinically the most severe, and only a slight fall in urinary 17-hydroxycorticoids and 17-ketosteroids followed administration of fluorohydrocortisone in the dosage of 1 mg. six-hourly. No effect was obtained by increasing the dosage to 2.5 mg. six-hourly. It is possible that still higher doses would have produced a significant effect, but Laidlaw *et al.* (1) failed to obtain a fall in 17-hydroxycorticoids or 17-ketosteroids in 3 cases of Cushing's syndrome treated with 10 mg. daily, and in 1 treated with 25 mg. daily. Cope and Harrison (6) also failed to obtain a response in total urinary steroids in 1 patient with Cushing's syndrome given 15 mg. daily, although these authors observed some depression in levels of urinary hydrocortisone alone. The technique required for the estimation of individual steroids is not, however, sufficiently developed for use as a routine test.

Very high dosages of fluorohydrocortisone are undesirable for two reasons; firstly, appreciable amounts may be excreted in the urine and so interfere with the estimation of endogenous 17-hydroxycorticoids, as found by Goldfien *et al.* (4); and secondly, the effect of the potent salt-retaining properties of this drug may be dangerous in a severe case of Cushing's syndrome with marked hypertension.

A failure to respond to fluorohydrocortisone could be due either to inability to inhibit a very great excess of corticotropin or to an unusually high sensitivity of the adrenal cortex to endogenous corticotropin, even in

diminished amounts. In Case 3, the recurrence of the disease following regrowth of the adrenal remnant, and the progressive pigmentation suggestive of increased secretion of melanocyte-stimulating hormone (intermedin) which is known to be closely associated with corticotropin (12), were indicative of excessive pituitary activity. It is conceivable that the amounts of fluorohydrocortisone given were not sufficient to overcome this hyperactivity. Whatever the mechanism may be, it seems that the degree of response to fluorohydrocortisone is related to the severity of the clinical picture. It is clear from the results obtained in Case 3 and from the experience of other workers that fluorohydrocortisone cannot provide unequivocal evidence for distinguishing adrenocortical hyperplasia from malignant tumor as a cause of Cushing's syndrome. Laidlaw *et al.* (1) regard the response to stimulation by corticotropin as a more valuable test, but the recent observation by Gallagher *et al.* (3) of an adrenocortical carcinoma responsive to corticotropin shows that it is necessary to be cautious when attempting to determine the pathogenesis of Cushing's syndrome prior to operation.

SUMMARY

1. Urinary 17-hydroxycorticoids and 17-ketosteroids have been studied in 3 cases of Cushing's syndrome due to adrenocortical hyperplasia.

2. The administration of corticotropin in 2 cases produced a considerable rise in 17-hydroxycorticoid excretion in both, but in only 1 was there a rise in 17-ketosteroids.

3. The administration of 9α-fluorohydrocortisone was followed by a fall in urinary 17-hydroxycorticoids and 17-ketosteroids in 2 cases, but in the third case there was no significant response.

4. It is concluded that the degree of response to fluorohydrocortisone in Cushing's syndrome is related to the severity of the clinical picture, and cannot be relied upon to differentiate adrenocortical hyperplasia from malignant tumor.

Acknowledgment

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EFFECT OF INTRAVENOUS CORTICOTROPIN ON THE LEVEL OF PLASMA HYDROCORTISONE IN MAN: APPLICATION TO THE ASSAY OF CORTICOTROPIN*

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IN THE course of a study of hydrocortisone metabolism in anxious human subjects (1), it was decided to measure their adrenocortical reserve (2). Several indirect methods have been employed for this purpose in the past, but recently a more direct method has been introduced, based on the determination of the increase in the plasma hydrocortisone level following the administration of corticotropin (3–6). Incidental to the major purpose of measuring the adrenocortical reserve of anxious subjects, we also wished to produce a graded series of responses to corticotropin in each subject. Prior to assessing the anxious subjects' adrenocortical capacity, we systematically explored the response of a group of healthy students to the administration of corticotropin. This involved measuring the plasma hydrocortisone level of each student before and during the administration of different doses of corticotropin given in varying order and with varying time intervals. A gratifying outcome of this study was the provision of a basis for relatively precise assay of corticotropin in human subjects.

SUBJECTS, EXPERIMENTAL DESIGN AND METHODS

Twenty-four college students, 20 men and 4 women ranging in age from 17 to 27 years (mean age, 22 years), served as paid volunteers in this study. Prior to participation, each subject was given a physical and psychiatric examination in order to rule out any condition for which corticotropin is contraindicated or which is known to alter adrenocortical function. Of 29 individuals examined, 5 were rejected as unfit for the experiment; the remaining 24 who were accepted were in good health.

The subjects were divided equally into two groups, which were equivalent in age and sex distribution. Each of the 12 members comprising the first group was tested on three different occasions spaced one week apart. Every subject was given 5, 10 and 15 U.S.P. units of corticotropin (ACTH) in randomized order, so that 2 individuals received each

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of the six possible permutations of the dose. This group will be called the "Randomized" group. The second group of 12 subjects ("Consecutive" group) also received the same three doses of corticotropin, but they were administered in consecutive order and spaced one day apart—5 units on day 1, 10 units on day 2, and 15 units on day 3.

The appropriate dose of Wilson's Corticotropin Solution (lot number 90664) was dissolved in 500 cc. of 5 per cent glucose solution and administered intravenously at a constant rate over a four-hour period beginning at about 9:30 A.M. A 25-cc. sample of blood was drawn from every subject on each occasion of testing just prior to, and one-half hour, one hour, two hours, and four hours after the administration of corticotropin was initiated. The plasma obtained was analyzed for hydrocortisone by the method of Nelson and Samuels (7, 8). The analysis of variance employed for the "Randomized" group has been described by Bliss (9); it permits the combination of two or more Latin squares. The log dose-response curves of the "Randomized" and "Consecutive" groups were compared by procedures outlined by Bennett and Franklin (10).

RESULTS

Variation in dose of corticotropin

The mean plasma hydrocortisone level and standard deviation for each group of subjects on every occasion of blood sampling and at each dose level of corticotropin are presented in Table 1. The corresponding changes from the pre-corticotropin value are given in Table 2. The changes from the pre-corticotropin level were compared for each dose by means of analysis of variance (Tables 3 and 4). A significant difference in response occurred within two hours after commencing the administration of corticotropin in both groups as a result of altering the dose. This difference in response was related to the dose in a linear fashion. Figure 1 demonstrates this relationship graphically for the change in plasma level at two hours. The linearity of the relationship was independent of the order of administration since it occurred in the "Randomized" group as well as in the "Consecutive" group. It is possible to distinguish the response to 5 units of corticotropin from that to 10 units as early as the first half hour after the commencement of administration; by contrast, a significant differentiation between the

TABLE 1. PLASMA HYDROCORTISONE LEVEL OF NORMAL SUBJECTS BEFORE AND DURING THE INTRAVENOUS ADMINISTRATION OF CORTICOTROPIN (ACTH)

Group	Dose of ACTH (U.S.P. units)	Plasma hydrocortisone level (μg./100 ml.)*				
		0 hr.	½ hr.	1 hr.	2 hrs.	4 hrs.
"Randomized"	5	12.0±4.5	20.1± 5.5	22.7± 4.4	27.4± 6.5	30.1± 9.8
	10	12.8±4.6	26.0± 5.4	28.5± 7.5	33.8±10.5	38.1± 9.6
	15	13.8±4.7	23.7± 9.1	29.5± 6.0	36.4± 9.6	43.3±17.5
"Consecutive"	5	13.5±6.2	22.8± 8.9	27.0± 9.8	31.6± 8.6	37.6±13.2
	10	12.3±6.3	25.1±11.0	31.4±14.3	40.6±11.4	46.7±14.6
	15	10.8±3.6	29.1± 7.7	30.8±13.4	44.7±10.3	47.8±20.2

* Mean ± Standard Deviation. Number of subjects in each group = 12.

TABLE 2. CHANGE IN PLASMA HYDROCORTISONE LEVEL OF NORMAL SUBJECTS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF CORTICOTROPIN (ACTH)

Group	Dose of ACTH (U.S.P. units)	Change in plasma hydrocortisone level from pre-ACTH level (μg./100 ml.)*			
		½ hr.	1 hr.	2 hrs.	4 hrs.
"Randomized"	5	8.11±5.44	10.67± 4.91	15.38± 6.51	18.02±10.44
	10	13.12±4.30	15.63± 7.20	20.98± 8.61	25.22± 8.14
	15	9.91±8.38	15.70± 6.62	22.58± 8.73	29.50±15.67
"Consecutive"	5	9.32±7.28	13.51± 6.97	18.06± 8.99	24.06±13.42
	10	12.79±8.42	19.07±11.65	28.33±11.95	34.46±15.10
	15	18.30±7.30	19.98±12.79	33.91±10.66	37.02±20.11

* Mean ± Standard Deviation. Number of subjects in each group = 12.

effects of 10 units and of 15 units did not occur even after four hours. The same results were obtained for the "Consecutive" group. This difference in response between 5 and 10 units on one hand and 10 and 15 units on the other hand, is due in part to the fact that the selected dosages of corticotropin were not in geometric progression. It would not have been advantageous to use 20 units of corticotropin instead of 15 units, for the maximum response (4) is evoked by 15 units; consequently the range of 10–20 units is not comparable to the range of 5–10 units. Unfortunately, the dose increments were selected prior to publication of any precise information about the log dose-response curve, and several compromises were made involving the dosages to be tested. Although the log dose is linearly related to the response for the group as a whole, considerable departure from linearity occurred in several subjects. Four of the 12 "Consecutive" group and 5 of the 12 "Randomized" group did not respond to an increase in dose

TABLE 3. ANALYSIS OF VARIANCE AMONG CHANGES IN PLASMA HYDROCORTISONE LEVEL AT DIFFERENT TIMES DURING ADMINISTRATION OF CORTICOTROPIN (ACTH), AND FOR VARIOUS DOSES—"CONSECUTIVE" GROUP

Source of variation	df	Change interval†							
		½ hr.		1 hr.		2 hrs.		4 hrs.	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Between subjects	11	101.98	2.71**	130.36	1.20	102.42	0.87	429.34	2.24*
Between doses	2	246.23	6.55***	147.33	1.35	775.60	6.60***	565.24	2.95*
(Linear)	[1]	484.20	12.89***	251.55	2.31	1507.34	12.83***	1007.51	5.27**
(Quadratic)	[1]	8.27	0.22	43.09	0.40	43.86	0.37	122.98	0.64
Error	22	37.57		108.76		117.44		191.35	
Total	35								

† For the change in level from pre-ACTH (0 hr.) to ½, 1, 2 or 4 hours after the beginning of ACTH administration.
* P significant at better than the 10% level of significance.
** P significant at better than the 5% level of significance.
*** P significant at better than the 1% level of significance.

TABLE 4. ANALYSIS OF VARIANCE AMONG CHANGES IN PLASMA HYDROCORTISONE LEVEL AT DIFFERENT TIMES DURING ADMINISTRATION OF CORTICOTROPIN (ACTH), AND FOR VARIOUS DOSES—"RANDOMIZED" GROUP

Source of variation	df	Change interval†							
		½ hr.		1 hr.		2 hrs.		4 hrs.	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Between subjects	11	40.90	0.97	36.74	0.90	94.65	1.89	184.14	1.54
(Between pattern)	[5]	41.67	0.99	45.39	1.12	18.56	0.37	53.74	0.45
(Between subjects in pattern)	[6]	40.26	0.95	29.53	0.73	158.06	3.16**	292.81	2.45*
Between trials	2	3.93	0.09	50.58	1.25	38.26	0.76	108.08	0.91
Between doses	2	77.23	1.83	99.85	2.46	171.39	3.42*	404.11	3.39*
(Linear)	[1]	19.44	0.46	152.01	3.74*	311.04	6.21**	791.20	6.63**
(Quadratic)	[1]	135.00	3.20*	47.69	1.17	31.73	0.63	17.01	0.14
Error	20	42.18		40.61		50.07		119.38	
Total	35								

† For the change in level from pre-ACTH (0 hr.) ½, 1, 2 or 4 hours after the beginning of ACTH administration.
* P significant at better than the 10% level of significance.
** P significant at better than the 5% level of significance.

with a progressively increasing response, as judged by the change in plasma level at two hours.

Variation in order of administration of doses

The order of administration of the doses of a drug can exert an influence on the response, in two different ways. The influence of the trial or occasion of drug administration, irrespective of the dose given, is one component of the order effect. Thus, the first trial might produce a greater response than the third trial, possibly due to a different psychologic reaction resulting from lack of familiarity with the experimental situation. The pattern of dose administration over several occasions (which in the present study was

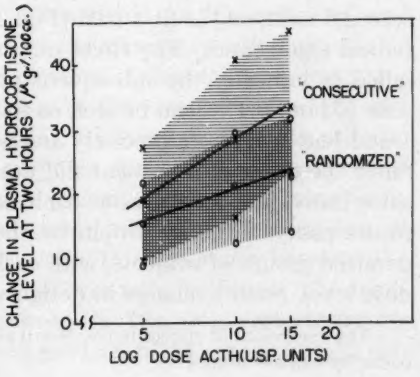

FIG. 1. The log dose-response curves of the "Randomized" and "Consecutive" groups. The upper and lower crosses at each dose level represent ±one standard deviation of the "Consecutive" group, whereas the upper and lower open circles represent ±one standard deviation of the "Randomized" group.

the six permutations of the three dose levels) is another component of the "order" source of variation in response to the drug. Priming effects on adrenocortical responsivity, which conceivably might vary with the amount of the preceding dose of corticotropin, might be the influential factor for this variable. It should be realized that trial variance is not completely free of pattern influence. For the "Consecutive" group, order was not considered separately, since it was confounded with dose; but for the "Randomized" group, it was possible to analyze separately each factor involved in variance due to order. The occasion of testing (trial) did not significantly effect the change in plasma hydrocortisone level assessed at each sampling time (Table 4). When the response to each dose of corticotropin was subdivided for the "Randomized" group according to the occasion of

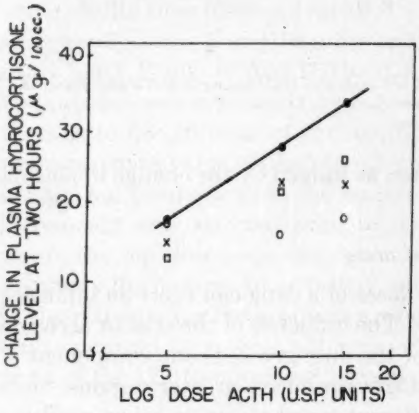

FIG. 2. The relation of log dose to response for the "Randomized" group when the response is subdivided according to occasion (trial) of corticotropin administration. The black circles represent the 12 members of the "Consecutive" group; the open circles represent the 4 members of the "Randomized" group receiving the dose on their first occasion of testing; the crosses represent the 4 members of the "Randomized" group receiving the dose on their second occasion of testing; and the squares represent the 4 members of the "Randomized" group receiving the dose on their third occasion of testing.

testing, the greatest responses for each subgroup of 4 subjects tended to occur on the occasion represented in the "Consecutive" group sequence, *i.e.*, the greatest response with 5 units occurred in those subjects who received 5 units on the first trial (Fig. 2). This tendency did not achieve statistical significance. The effect of trial on response was minimal and also failed to influence the subsequent pre-administration level of hydrocortisone (0 hour). This can be seen on inspection of the 0-hour values in Table 1 and has been more precisely assessed by means of analysis of variance. Since the order of treatment did not significantly influence response, and since individual responses at each dose level were not highly correlated,[1] future assays of corticotropin by the present method might employ randomized groups of subjects, with each group receiving corticotropin at one dose level. Such a change in design would greatly speed up the collection

[1] The coefficient of concordance, $W = 0.40$; for 11 degrees of freedom, this result could occur by chance alone (11).

of the data with little loss in precision, since only one occasion of testing for each subject would be required.

Although estimation of the effect of occasion of corticotropin administration furnished considerable information about the influences of order of treatment on response, it did not account for any effects which might be exerted by a previous treatment. In order to get an estimate of this "pattern" variance, we partitioned the *between-individual variance* into two components—one due to *between-pattern variance* and the other to *between-subjects-in-pattern variance.* The influence of pattern on response at each sampling time was found to be negligible (Table 4). The results of the trial and pattern sources of variance indicate that order can be neglected as a factor contributing significantly to the total variation of response to corticotropin.

Variation in the time interval between doses

The subjects of the "Randomized" group were given three doses of corticotropin spaced a week apart, whereas the members of the "Consecutive" group received their doses a day apart. Several investigators have cautioned against repeating a corticotropin response test in the same subject too closely in time. Bliss *et al.* (3) do not repeat an assay in less than a week; Renold and coworkers (12) in less than three days; and DiRaimondo and his associates (13) in less than four days. In order to determine whether the shorter time interval produced a greater response at each dose level, Figures 1 and 2 were reinspected. In Figure 1, the "Consecutive" group curve is displaced upward from the "Randomized" group, but in Figure 2 the open-circled values of the "Randomized" group approach the closed-circled values of the "Consecutive" group, *i.e.*, the subjects receiving treatment corresponding to that of the "Consecutive" group resemble that group to the greatest degree. In order to test the hypothesis that the time interval between dose administration did not influence response, the two lines representing the dose-response relationship of the "Consecutive" and "Randomized" groups were compared by the standard methods available for this purpose. These included a comparison of the standard deviations about the computed lines (σ_{xy}), the slopes of the computed lines (b) and the intercepts of the lines on the Y-axis (a). By every one of these criteria, the differences between the two dose-response curves did not achieve the 5 per cent level of confidence. However, the mean change in hydrocortisone level was greater for the "Consecutive" group in 11 of the 12 occasions of measurement. On the majority of occasions, this difference between the two groups with respect to change in level was not significant. The tendency toward difference in response between the two groups was tested by an analysis of variance designed to compare trends (14). A significant dif-

ference between the groups occurred only for the change in level at two hours. On the other sampling occasions, although the "Consecutive" group manifested greater response, the values were not very different from those of the "Randomized" group. Since identical results were not obtained by the two methods, conclusions are somewhat difficult. However, in view of the observations of other investigators that a progressive enhancement of adrenocortical responsivity occurred when the time between repetitive administrations of corticotropin was diminished, the "Consecutive" group was expected to respond to corticotropin with a somewhat greater rise in the level of plasma hydrocortisone. Consequently we are inclined to weight the "trend analysis" results more heavily than the other criteria of dose-response differences. Furthermore, in the "Consecutive" group, the highest dose of corticotropin on the third occasion of testing produced a response 50 per cent greater than did 15 units of corticotropin in the "Randomized" group, whereas at the lowest dose on the first occasion the response was only 17 per cent greater. This finding also suggests that a priming effect on the adrenal was occurring in the "Consecutive" group, due to the time interval between dose administrations.

Precision of the corticotropin assay

The data of the present study provided two complete analyses of the same lot of corticotropin. From these results one may determine various assay characteristics. The most frequently reported and useful statistic is the index of precision (λ), which was calculated for each analysis and is presented in Table 5 with the value obtained by Bliss *et al.* (3) using a similar assay technique. Our index of precision of 0.33 obtained for the "Consecutive" group compares favorably with those reported elsewhere.

In an earlier section, we mentioned that several individuals of each sub-

TABLE 5. SUMMARY OF ASSAY STATISTICS FOR CORTICOTROPIN USING THE PLASMA HYDROCORTISONE LEVEL CHANGE AT 2 HOURS AS THE RESPONSE

Group	Standard deviation about line (σ_{xy})*	Slope of line (b)	Index of precision (λ)
"Randomized"	7.07 (6.43)†	15.5 (28.6)	0.46 (0.224)
"Consecutive"	10.84 (7.90)	33.3 (51.0)	0.33 (0.155)
Bliss *et al.* (3)	7.61	11.5	0.66

* Derived from the error term of the appropriate analysis of variance rather than from direct computation based on the assumption of linearity of the log dose-response relationship (Bliss (9)).

† The values in parentheses are for the 7 selected "Randomized" and 8 selected "Consecutive" subjects.

group did not respond to an increase in the dose of corticotropin with a progressively increasing response. Actually, these subjects' plasma levels rose only slightly or even fell. Figure 3 shows the dose-response relationship for every subject in the "Consecutive" group. Results for those persons who differed markedly with respect to adrenocortical responsivity were discarded and the data re-evaluated using only the most identical subjects. Such selection tends to distort the response of a group chosen by *a priori* considerations and is obviously prohibited in the establishment of normal values. However, in addition to determining the responses of a group of healthy students to corticotropin, this study permitted the investigation of an assay technique. Since increased uniformity of subjects yields increased precision in the assay and since it is impossible to identify the unusual responders prior to the experiment, it is permissible to discard the deviant responses. DiRaimondo and his coworkers (13) employed a comparable procedure to improve their index of precision. The recalculation markedly improved the index of precision as a result of the large increase

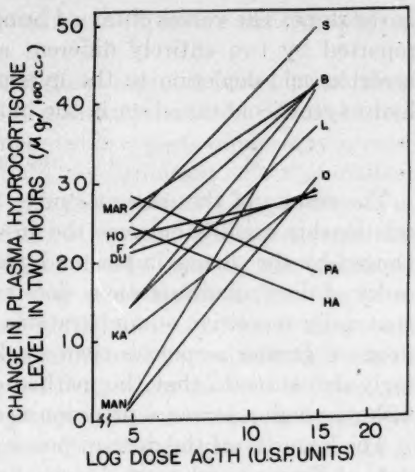

FIG. 3. The individual log dose-response curves of the subjects of the "Consecutive" group. Note the difference in slope for the 4 Subjects Pa, Ha, Du and Ho compared with the other 8 subjects.

TABLE 6. THE INDEX OF PRECISION (λ) OF THE LOG DOSE-RESPONSE LINE FOR CORTICOTROPIN OBTAINED BY THREE DIFFERENT METHODS OF ASSAY

Method	λ
Adrenal ascorbic acid-depletion in hypophysectomized rat*	0.14 (0.12–0.16)
Urinary hydroxycorticoid excretion (increase) in human subject†	0.18 (0.06–0.29)
Plasma hydrocortisone level (increase) in human subject‡	0.19 (0.15–0.22)

* Mean of 2 different assays; figures in parentheses show the range of values.
† Mean of 7 different assays.
‡ Mean of 2 different assays.

in the slope. The values obtained bring our results closely in line with those reported by two entirely different assay methods, namely, the adrenal ascorbic acid-depletion in the hypophysectomized rat (15), and urinary hydroxycorticoid excretion in the human subject (13) (Table 6).

DISCUSSION

The results of the present study: 1) confirm the hypothesis of a linear relationship existing between the dose of corticotropin and the response as gauged by the change in plasma hydrocortisone level, 2) indicate that the order of dose administrations does not greatly alter response, 3) suggest that daily repetitive administrations of corticotropin to the same subject produce greater responses than weekly administrations, and 4) convincingly demonstrate that the method of corticotropin assay employed furnishes as high a degree of precision as any other method presently available.

The linearity of the dose-response relationship is fully evident from the data of Figure 1 and, more directly, from the analyses of variance in Tables 3 and 4. Indeed, after elimination of the subjects who differed markedly with respect to adrenocortical responsivity, the variance due to dose accounts for about 50 per cent of the total variance as estimated from the sum of squares. Because of the linear relationship and the steep slope of the relationship, small differences in corticotropin concentration (0 to 5 U.S.P. units) are readily detectable. The assay is comparable to the method of DiRaimondo and coworkers (13) with respect to its sensitivity; however, with the hypophysectomized rat adrenal ascorbic acid-depletion technique (15) far smaller quantities (0.000 to 0.003 U.S.P. unit) can be detected.

The lack of effect of either component (trial, pattern) of order of dose administration on response is of considerable practical importance. The design of a corticotropin assay can be considerably simplified as a result, by the elimination of repetitive testing in the same subject. Analysis of our data revealed that differences between individuals did not significantly affect response, although, as Liddle (16) pointed out, individuals tend to maintain their same position relative to the group on repetitive testing. This results in a slight improvement in the index of precision. However, by proper selection, subjects who closely resemble one another with respect to adrenocortical capacity may be obtained, and thus the need for repetitive testing on each individual is obviated. Proper selection may be achieved by testing a group of subjects at two different dose levels and employing only those with similar response slopes for subsequent corticotropin assays. This would make for greater speed in obtaining results and avoid the difficulties of frequent venipuncture with human subjects. The absence of significant between-subject variance suggests that "cross-over" design is not essential for great precision. As a result, this corticotropin

assay can become as simple in design as that employing the hypophysectomized rat.

In analyzing the influence of order of dose administrations on response, we assessed the effect of pattern as a subsidiary variable. The statistical evaluation of pattern is usually not included in reports of bioassay investigations. Although in the present study no significant effect of pattern emerged, mention has been made of the procedure as a suggestion of an additional approach in the analysis of bioassay data. It is conceivable that such effects may become apparent with other substances by this technique.

Although our statistical procedures were more involved than the situation warranted, conclusions regarding the differences between the groups are not as convincing as they would have been had we obtained the same results by the two methods of analysis. Both a comparison between the "Consecutive" and "Randomized" groups on each occasion separately, and expectation based on the findings of other investigators, induce us to give preference to the trend analysis. However, we consider it appropriate to report that a different type of statistical treatment leads to a conflicting conclusion.

The indices of precision obtained for our total sample are comparable to, or better than those reported in the literature. In comparison with the data of Bliss (Table 5), our greater precision resulted from a considerably steeper slope and not from a reduced standard deviation about the dose-response line. Despite the fact that the pre-corticotropin hydrocortisone levels in his subjects were almost identical to ours, the magnitude of the responses was considerably less. Our subjects constituted an extremely homogeneous group with respect to age, physical condition, mental status and educational level. The careful selection technique which we employed avoided to a considerable degree the pitfalls involved in the use of volunteer research subjects (17). However, assuming that our subjects were more homogeneous than those of Bliss, one would expect that the contribution of lesser subject variance toward a better index of precision would be in a reduction of the standard deviation about the dose-response line rather than in an increase of the slope. In another study in which we determined the adrenocortical reserve of a group of hospital personnel (doctors, nurses, technicians) whose age range was comparable to that of Bliss' subjects, we obtained a lesser response to 15 units of corticotropin than in either of the student groups. We do not believe that this difference was due to age alone. Although we lack conclusive evidence to support the view that the student group was considerably more anxiety-prone than the hospital employee group and hence more responsive to stimulation with corticotropin (2), this hypothesis warrants further investigation.

The method employed in this study for the assay of corticotropin, in

which the drug was administered intravenously to human subjects and its effects estimated by the subsequent rise in the plasma hydrocortisone level, yielded an index of precision which was about as good as that obtained by the estimation of urinary hydroxycorticoids (13) or the depletion of ascorbic acid in the adrenal of the hypophysectomized rat (15). DiRaimondo obtained a lower index of precision by selection of subjects on the basis of uniformity of slope; when we selected our subjects by the same principle, our index of precision became almost identical. It might have been anticipated that intravenous assay would be superior to the extravascular method, since the drug is supplied directly into the blood stream and the adrenocortical response is determined by measurement of the circulating hormone of the adrenal cortex (18, 19). With the intramuscular procedure, considerable loss of activity may occur as a result of the indirect route of administration, and the response is not as direct an estimate of adrenocortical function as is the plasma hydrocortisone level. Likewise, it might have been expected that Sayers' method (15) would be superior to intravenous assay, since the rat method employs a far more homogeneous population sample and the response index (depletion of adrenal gland ascorbic acid) is more closely related to the functional activity of the adrenal cortex than is a change in the peripheral vascular hydrocortisone level. Despite these tentative expectations, the three assay methods yield similar degrees of precision. Numerous arguments can readily be advanced in favor of, or against each of the three procedures. The intravenous corticotropin method does offer certain advantages over the other two assay techniques: 1) the test material is delivered in the most direct fashion to its target cells, 2) the test material is assayed in the animal for which it is primarily intended, and 3) the estimate of its effectiveness is the most direct reflection of adrenocortical activity possible in man. However, it should be noted that corticotropin is frequently administered intramuscularly in a slow-acting vehicle and consequently estimates of corticotropic activity derived by the intravenous technique might be unsuitable if the preparation is normally given by an extravascular route. This difficulty might be overcome by combining the best features of each assay procedure—extravascular administration of corticotropin and estimation of the plasma hydrocortisone level.

SUMMARY

The intravenous administration of from 5 to 15 U.S.P. units of corticotropin to human subjects and the subsequent estimation of its action on the adrenal cortex by means of the determination of the plasma hydrocortisone level provides a useful method for the assay of corticotropin. The order of administration of differing dosages of corticotropin does not con-

tribute significantly to the response. The precision obtained by this method equals those obtained by the urinary hydroxycorticoid assay and the adrenal gland ascorbic acid-depletion assay. Several statistical techniques employed for the purpose of comparison of treatments may be of some value for other drug assays.

Acknowledgments

Drs. Melvin Sabshin and Francis A. Board conducted the psychiatric interviews which contributed to the screening of the subjects and the homogeneity of the group. Dr. Herbert Silverstone gave valuable suggestions with respect to the statistical analyses. Miss Betty Lou Day assisted with the chemical determinations and the statistical computations.

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SPORADIC HYPOTHYROIDISM ASSOCIATED WITH GOITER*

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THE occasional association of goiter with hypothyroidism in non-endemic areas has been known for many years. However, it was not until 1943 that Hamilton, Soley, Reilly and Eichorn (1) first reported functional studies using radioactive iodine in 2 hypothyroid patients with goiter. In 1946 Lerman, Jones and Calkins (2) reported studies on 2 sporadic cretinous brothers with goiters. In 1950 appeared Stanbury and Hedge's (3) now classic studies on 3 of 4 goitrous cretins in a single family. Subsequently Stanbury and his associates (4–7) have contributed much to the understanding of the disturbance in thyroid synthesis in these patients. Hubble (8), McGirr and Hutchison (9, 10), Wilkins *et al.* (11), Jackson (12), Zondek *et al.* (13), Burrell and Gairdner (14), and Levy *et al.* (15) have also reported various clinical features and functional studies in patients with thyroid enlargement associated with hypothyroidism.

It is now generally accepted that these patients have a defect in synthesis of thyroid hormone. In some of the reports this has been documented beyond any reasonable doubt (3–7). Since the defect may be in any one step of the complex chain of reactions which lead to the final elaboration and discharge of the thyroid hormone, one would predict that many different functional types of goitrous cretins would occur. And indeed, this has been amply demonstrated by the investigations of Stanbury and associates (3–7).

The present investigation concerns studies of thyroid function in a child with a goiter and hypothyroidism.

CASE REPORT

First admission

A.U., a 7½-year-old white female child, was admitted to St. Christopher's Hospital for Children for the first time in June 1954 because of an enlarged thyroid. According to

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her mother, the enlargement had been present since 3 years of age and perhaps longer. During that time the thyroid enlargement allegedly varied in size, occasionally being barely perceptible. However, owing to persistence of the enlargement, the child was brought to the York Hospital Pediatric Clinic, York, Pennsylvania, by a State Health Nurse.

At that time (February 1954) the girl was 44 inches tall and weighed 38 pounds. A blood count and results of urinalysis were normal, and the serum cholesterol level was 172 milligrams per 100 ml. Her bone age was said to be that of a child aged 1½ to 2 years. In March 1954, therapy was started with Lugol's solution, 3 drops three times daily. As the goiter did not decrease in size, this therapy was discontinued on May 5, 1954. In April 1954, the BMR was +9 per cent. On April 7, thyroid therapy with Proloid, 15 mg. daily, was instituted. Both Lugol's solution and Proloid were discontinued on May 5, 1954. On May 6 the serum level of cholesterol was 228 mg. per 100 ml. On June 6, 1954, she was admitted to St. Christopher's Hospital for Children for further studies.

Additional history revealed that the child's mother had never married. Nothing was known of the father. The patient had an 18-year-old sister who was healthy, married and had a healthy infant. The mother was one of 14 siblings; one died in infancy of an unknown cause, but in none of the others was there any known abnormality. The maternal grandmother had a "large goiter" and died at the age of 48 years.

The patient was born and lived all of her life in the vicinity of York, Pennsylvania, a region where goiter is uncommon. The delivery was uneventful, and the birth weight was 7½ pounds. She was jaundiced in the neonatal period but other details are not known. She had always been "constipated," even as an infant.

FIG. 1. Patient A.U., at 8 5/12 years. Note moderate sized goiter but little overt evidence of hypothyroidism.

According to the history, developmental progress had been within the limits of normal. Except for frequent infections of the respiratory tract, she had had no other illnesses. She had always been "active" and was doing well in the first grade in school.

Dietary history revealed that the girl had always been a "poor eater." She disliked all "greens," and no history of excessive intake of goitrogenic foods was elicited. Although the family used well-water occasionally, the usual supply was from a municipal source.

Physical examination. The patient was a pleasant and cooperative girl with a mild speech defect and somewhat limited intelligence. She weighed 38¼ pounds and was 44 inches in height. Respirations were 20 per minute, and the pulse varied from 80 to 118 per minute. The systolic blood pressure was 98 mm. Hg and the diastolic, 60. The thyroid gland was moderately enlarged and was smooth and soft with no palpable nodules (Fig. 1). Neither a bruit nor a thrill was present. The skin was soft, smooth and warm, and the hair was fine. The tonsils were moderately hypertrophied. Examination of the heart, lungs and abdomen revealed no abnormalities. The genitalia were normal for the chronologic age.

Laboratory data (Table 1). The hemoglobin level

TABLE 1. LABORATORY DATA

Date	Age (yrs.)	Ht. (ins.)	Ht.-Age (yrs.)	Bone age* (yrs.)	Serum			Thyroidal I^{131} uptake at 24 hrs. (%)
					Cholesterol (mg./100 ml.)	Alk. phosphatase (B.U.)	PBI (μg./100 ml.)	
Feb. 1954	$7\frac{6}{12}$	44	$5\frac{6}{12}$		172			
June 1954	$7\frac{8}{12}$	44	$5\frac{6}{12}$	$2\frac{3}{12}$	290	1.2 1.6	7.7 9.6	64
June 1955	$8\frac{8}{12}$	46	6	$3\frac{9}{12}$	334	0.8	3.0 4.9	58
Nov. 1955	$8\frac{10}{12}$				208	2.7		
Dec. 1955	$8\frac{11}{12}$	48	7	$5\frac{8}{12}$			7.7	

* Todd's standards.

was 11.6 grams per 100 ml. The white blood cell count was 10,000 per cu. mm., with 70 per cent neutrophils, 24 per cent lymphocytes, 5 per cent monocytes and 1 per cent basophils. Urinalysis did not reveal any abnormality; the specific gravity was 1.025. The serum level of total cholesterol was 290 mg. per 100 ml., and of cholesterol esters 184 mg. The serum level of calcium was 11.5 mg., of phosphorus 3.21 mg., and of alkaline phosphatase 1.2 and 1.6 Bodansky units per 100 ml. on successive occasions. An electrocardiographic tracing was within the normal range. The Stanford-Binet intelligence test indicated an I.Q. of 78, and the Weschler Bellevue intelligence scale for children showed a performance I.Q. of 86, a verbal I.Q. of 80, and a full scale I.Q. of 81.

The speech therapist stated that there was no involvement of the speech mechanism, and felt that the defect was related to the mental retardation.

Roentgenologic studies revealed a bone age of 2 years and 3 months by Todd's standards; it was in the tenth percentile for a child of $2\frac{1}{2}$ to 3 years of age by Voght and Vickers' standards. The osseous pattern and mineralization were otherwise not abnormal. Swallowing function, as observed by a barium-water mixture during fluoroscopic examinations, was within the range of normalcy. The basal metabolic rate was +24 per cent. The serum protein-bound iodine level was 7.7 and 9.6 micrograms per 100 ml. on successive determinations.

On June 11, 1954, a tracer dose of 23 microcuries of radioactive iodine was given orally. The retention of radioiodine by the thyroid gland at twenty-four hours was 64 per cent.

Chromatographic studies. On July 8, 1954, the patient was given 200 microcuries of radioactive iodine. Plasma was collected after twenty-four and forty-eight hours and was examined by Dr. Jack Gross at the State University of New York College of Medicine at New York City. The plasma was chromatographed on paper in a butanol-dioxane-ammonia system using previously described techniques (16). His report follows: "We have found that the PBI^{131} was 66 and 75 per cent for the 24 and 48 hour samples, respectively. On analyzing this material further, it was found that only about 15 and 37 per cent for the two samples, respectively, was extractable with butanol and identifiable as thyroxine on the chromatograms (Fig. 2). The remaining PBI^{131} was resistant to extraction after prolonged treatment with butanol and only liberated its iodine after some hydrolysis."

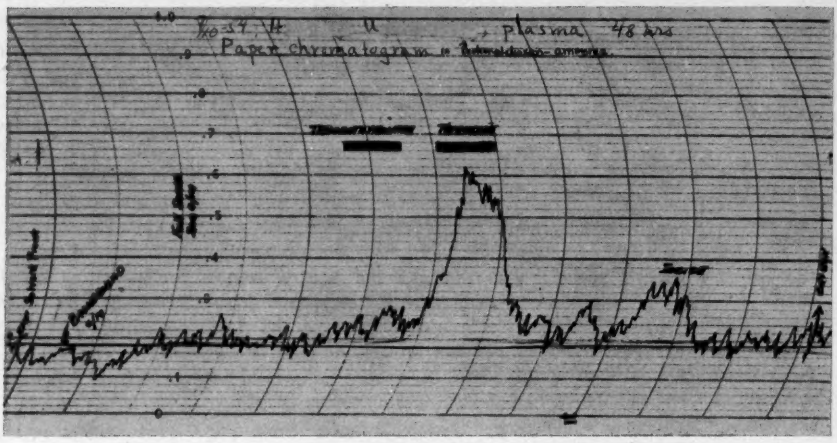

FIG. 2. Paper chromatogram made forty-eight hours after administration of radioiodine. The solvent system was butanol-dioxane-ammonia and the full scale represents 200 counts per minute. Note the peaks for iodide and thyroxine and a suggestion of one for triiodothyronine.

As the patient showed little disability related to the mild hypothyroidism, it was decided to defer therapy temporarily.

Second admission

The patient refused readmission for further studies until June 1955, at which time she was 8½ years of age.

There had been no remarkable changes in the physical findings. She was 46 inches in height and her weight was 43½ pounds. The goiter was essentially unchanged. She had been promoted to the third grade in school and was receiving good marks in her studies. She had received no medication whatsoever during the previous year.

Laboratory studies (Table 1). The hemoglobin level was 10.9 Gm. per 100 ml., the white blood cell count was 8900 per cu. mm., with 51 per cent neutrophils, 48 per cent lymphocytes and 1 per cent basophils. The urine was normal, with a specific gravity of 1.011. A serologic test for syphilis yielded negative results. The level of serum calcium was 11.7 mg., of phosphorus 4.97 mg., of alkaline phosphatase 0.8 Bodansky unit, and of cholesterol 334 mg. per 100 ml. The concentration of fasting blood sugar was 78 mg., and of urea nitrogen 11 mg. per 100 ml. The serum protein-bound iodine level was 3.0 and 4.9 micrograms per 100 ml. on successive occasions.

Her I.Q. with the Stanford-Binet test was scored at 78. The electroencephalogram and the electrocardiogram were within normal limits. An audiometric test did not disclose any abnormality in hearing. Roentgenologic study of all the epiphyses revealed a bone age of 3 years and 9 months according to Todd's standards; it was in the tenth percentile for a child 3½ to 4 years old by Voght and Vickers' standards. Roentgenograms showed the skull and chest to be within normal limits. The dental age was estimated to be 8½ years.

Radioiodine studies. On June 16, 1955, the patient was given 40 microcuries of radioactive iodine intravenously. Measurements were made using a bismuth cathode tube

with a side-window. The thyroidal uptake was determined at twenty-five, twenty-five and a half, and thirty hours at distances of 25 and 30 centimeters. The averages of the measurements at these two distances were used. Immediately after the measurement at twenty-five hours, the patient was given 1 gram of potassium thiocyanate by mouth. At the end of twenty-five hours, the radioiodine accumulation in the thyroid gland was 58 per cent. Half an hour after administration of the potassium thiocyanate the radioactivity over the thyroid gland was 57 per cent, and five hours later it was 51 per cent of the administered dose.

Two hours after injection of the radioiodine, the level of protein bound I^{131} was 0.13 per cent of the administered dose per liter of serum. The concentration of protein-bound I^{131} at twenty-five hours was 0.58 per cent of the administered dose per liter of serum. The 24-hour cumulative urinary excretion was 17.3 per cent of the administered dose.

Thyrotropic-hormone (TSH) assay. Serum obtained during the first admission was immediately frozen for subsequent assay. The stasis tadpole bioassay technique of D'Angelo was used (17). The mean height of the thyroid cells of the test animal was 5.10 ± 0.13 micra. This value is within the range obtained for the control stasis tadpole and indicates absence of TSH activity (18).

Subsequent course. On June 18, 1955, therapy with 30 mg. of desiccated thyroid per day was instituted, and the dose was gradually increased to 90 mg. daily. At York Hospital in November 1955 the level of serum cholesterol was 208 mg., of alkaline phosphatase 2.7 Bodansky units, and of phosphorus 5.2 mg. per 100 ml. The serum protein-bound iodine level was 7.7 micrograms per 100 ml. (Table 1).

The patient was next seen in December, 1955. Her mother had noted little change in her except that the goiter had disappeared. She was as active as usual and was getting good grades in school. She was 48 inches in height, and weighed 44½ pounds. The only change on physical examination was the marked reduction in the size of the thyroid gland. Although the gland was still palpably enlarged, the enlargement was no longer visible. Roentgenograms of the bones showed an osseous age of 5 3/12 years by Todd's standards; it was in the tenth percentile for a 5½-year-old child by the standards of Voght and Vickers.

DISCUSSION

This patient demonstrated a goiter with evidence of mild but indisputable hypothyroidism. In support of hypothyroidism was the retarded physical, mental and osseous development, the slightly elevated serum cholesterol level and the depressed serum alkaline phosphatase level. These were present in association with an elevated concentration of serum protein-bound iodine and elevated radioiodine accumulation by the thyroid gland.

After an interval of one year without therapy there had been little change in the clinical status of the patient or in the size of the goiter. There had been a further decrease in the level of alkaline phosphatase, a slight increase in the level of serum cholesterol and a decrease in that of protein-bound iodine. An abnormally high thyroidal accumulation of radioiodine, an elevated serum protein bound I^{131} concentration, and only a small discharge of the thyroid-trapped iodine following orally administered potassium thiocyanate were demonstrated.

The chromatographic studies showed that only a small proportion—

15 per cent of the serum protein-bound iodine at twenty-four hours, and 37 per cent at forty-eight hours—was butanol-extractable and identifiable as thyroxine. It appears, therefore, that this child's thyroid could trap and bind iodine, and that she was in fact releasing some thyroxine. However, only a fraction of the circulating hormone was thyroxine. The remainder of the material appeared to have been a biologically less potent iodinated substance, as judged by the clinical findings of hypothyroidism. The exact nature of this substance was not determined in this patient. No assumptions can be made concerning the nature of this material, except that it is not free thyroxine, triiodothyronine, monoidothyrosine or diiodotyrosine. However, any or all of these substances may have been present as integral amino acids in combination with protein.

Further confirmation of the diagnosis of hypothyroidism was obtained by the therapeutic response. Administration of thyroid hormone resulted in accelerated growth, epiphyseal maturation and depression of serum cholesterol concentration to a normal level.

The data obtained in this patient differ from those in previously reported cases of hypothyroidism attributed to defective synthesis of thyroid hormone. They are, however, reminiscent of data obtained in patients with the Hashimoto type of thyroiditis. Gribetz, Talbot and Crawford (19) reported data in 6 children with chronic lymphocytic thyroiditis. One of the children demonstrated an elevated thyroidal uptake of radioiodine, and 3 children had abnormally elevated serum levels of protein-bound iodine (PBI). In the latter 3, butanol-extractable iodine (BEI) levels were also measured; they were within the lower range of normal. These authors suggested that in thyroiditis the follicles release either partially proteolyzed or unproteolyzed thyroglobulin or noncalorigenic compounds similar to diiodotyrosine, or both, into the circulation. More recently, Skillern and associates (20) reported clinical studies on a group of patients with struma lymphomatosa. Many of them demonstrated clinical evidence of hypothyroidism in addition to a low basal metabolic rate and an elevated serum cholesterol level. The serum PBI concentration was in the lower range of normal. Some of the patients with a normal PBI level had a low BEI level. The average disparity between the PBI and BEI values in 10 patients was 1.8 μg. These authors suggested that this disparity could be due to the presence of a metabolically inactive iodinated protein. The 24-hour thyroidal I^{131} uptakes varied from 5 to 98 per cent. It is interesting that the patient with a 24-hour thyroidal I^{131} uptake of 98 per cent was a 12-year-old girl with a goiter of two years' duration. She was the only child in this series of cases. Her serum PBI level was 5.1 mg. per 100 ml., and BEI level 2.5 mg. per 100 ml.

Skillern *et al.*, suggest that struma lymphomatosa is primary thyroid

failure with compensatory thyroid enlargement. Thus, the lymphocytic infiltration and fibrosis in these goiters are probably secondary to the failure of the thyroid cells, rather than the cause of this failure.

More recently, Owen and McConahey (21) reported chromatographic studies on patients with Hashimoto's thyroiditis. They indicated that many of these patients have a normal or elevated serum protein-bound iodine level, and thyroidal radioiodine accumulation in the "hyperthyroid" range in the presence of a reduced basal metabolic rate. They reported that as much as half of the serum PBI was not extractable with acidified butanol in such patients. They also suggested that the pathologic process of Hashimoto's thyroiditis may lead to release of thyroglobulin directly into the blood. Kieselguhr column chromatography of trypsin-hydrolyzed glands of patients with Hashimoto's thyroiditis who have received I^{131} revealed a "fairly normal" distribution of radioactive thyroxine, triiodothyronine, iodine, diiodotyrosine and monoiodotyrosine (21).

Whitelaw, Thomas and Reilly (22) recently reported studies on a nongoitrous, $8\frac{1}{2}$-year-old cretin with a high serum PBI level and high thyroidal I^{131} uptake. The serum cholesterol concentration was 370 mg. per 100 ml., the BMR −18 per cent, and the serum PBI levels 11.2 and 10.3 micrograms per 100 ml. Thyroidal I^{131} uptake was 100 per cent, with a conversion ratio of 72 per cent. Chromatographic studies showed a large fraction in the form of an unknown iodoprotein which remained at the origin. There was failure of radioiodine discharge with potassium thiocyanate. The similarity of the findings in this patient and in the one reported here are striking. Whitelaw *et al.* did not record the histologic appearance of the gland.

It is impossible to draw any conclusions concerning a possible relationship of the thyroid dysfunction to lymphoid thyroiditis in the case of Whitelaw *et al.* and in our case. Studies thus far reported suggest that these patients have defects in the synthesis or in the release of thyroid hormone, or in both processes. They may release nonproteolyzed iodinated protein, possibly globulin. However, the unknown iodinated protein has as yet not been unequivocally identified as thyroglobulin. If it could be ascertained that the same functional disturbance occurs in patients with and without histologic evidence of so-called "lymphoid thyroiditis," this would be confirmatory evidence for the thesis proposed by Skillern *et al.* (20) that the "lymphoid thyroiditis" is secondary to the "thyroid failure" or, more specifically, secondary to defective synthesis or release of thyroid hormone. The question would then be raised as to why some patients do, and others do not exhibit the lymphoid pattern in the thyroid gland.

Failure to demonstrate TSH activity in our patient is difficult to explain according to our current concepts of thyroid physiology. It is generally assumed that the goiter of goitrous cretins is secondary to elevated levels

of TSH. It is interesting that Stanbury and colleagues found normal levels of TSH in an adult male with a congenital goiter (6). The assay was performed when the patient was 27 years of age and at a time when the goiter was enlarging. Postel has produced some evidence demonstrating non-iodine-containing inhibitors in human serum (23). Although this is a possible explanation of the failure to demonstrate TSH activity in our patient, additional data are needed before conclusions are justifiable. He used zone electrophoresis to fractionate the serum and then bio-assayed the fractions, using the radioactive iodine uptake by the chick thyroid gland as an index of TSH activity.

CONCLUSIONS

A child with clinical and laboratory evidence of hypothyroidism in association with a goiter is described. Accumulation of radioiodine in the thyroid and protein binding of I^{131} in the serum were in the hyperthyroid range. Only a third of the serum PBI^{131} was extractable with butanol and identifiable chromatographically as thyroxine. The significance of the findings as related to previously described defects of synthesis or release of thyroid hormones is discussed.

Addendum

We have studied another patient at 12 months of age who showed signs and symptoms of moderately severe hypothyroidism. The thyroid gland was not palpably enlarged. The serum protein-bound iodine level was 3.0 micrograms per 100 ml. At twenty-four hours the thyroidal uptake of radioiodine was 36 per cent and the concentration of protein-bound I^{131} was 1.0 per cent of the administered dose per liter of serum. Only 50 per cent of the radioactivity in the serum was extractable with butanol. Satisfactory chromatograms were not obtained because of the low level of radioactivity in the serum

Acknowledgment

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THE METABOLISM OF IODOTYROSINES. IV. METABOLISM OF *l*-DIIODOTYROSINE IN PATIENTS WITH HYPOTHYROIDISM

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WHEN diiodotyrosine labeled with radioactive iodine is administered to normal subjects, almost all of the labeled iodine which appears in the urine is in the form of inorganic iodide. Albert and Keating (1), observing the disposal of labeled diiodotyrosine in 3 patients with treated myxedema, found that only approximately 5 per cent appeared in the urine as diiodotyrosine. The optical activity of the diiodotyrosine was not stated. Entirely comparable results have been obtained in 15 euthyroid patients with various diseases (2). When labeled racemic (*dl*) diiodotyrosine was injected intravenously into 3 patients with hypothyroidism and goiter, however, the labeled iodine which appeared in the urine was in the form of diiodotyrosine (3). No deiodination of diiodotyrosine could be demonstrated. Identical findings were obtained in 2 of these patients after they had been treated with desiccated thyroid.

In the present study, the urinary disposal of levorotatory diiodotyrosine has been observed in a group of patients without thyroid disease and in patients with hypothyroidism and with clinical myxedema. The results are compared with those obtained from administration of racemic diiodotyrosine to euthyroid subjects.

METHODS

The patients were selected from the wards and the ambulatory clinic. Labeled diiodotyrosine was administered intravenously in 8-ml. to 10-ml. volume. Urine samples were collected without catheterization at time intervals as shown in Table 1.

Labeled diiodotyrosine was prepared by iodination of *l*-tyrosine at room temperature, by a modification of the method of Roche *et al.* (4). The preparation for injection was prepared by elution of the appropriate zone after chromatographic resolution on Whatman #3 paper in a butanol-acetic acid solvent system. The preparation of labeled *l*-diiodotyrosine used in Patients 9 and 10 (Table 1) was prepared by exchange with *l*-diiodotyrosine.

Analysis of urine specimens was as previously described (2). Descending phase chromatography in a butanol-acetic acid solvent system was used throughout. The

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TABLE 1. THE EXCRETION OF LABELED IODINE AFTER ADMINISTRATION OF I^{131}-LABELED DIIODOTYROSINE (*l*-T_2*)

Patient			Diagnosis	Serum PBI (μg./100 ml.)	Carrier *l*-T_3	Labeled T^*_3 in dose (%)	% Dose excreted			% Labeled iodine in urine as labeled T_3*			% Dose labeled T_3* excreted as T_3*		
No.	Age	Sex					0–1 hr.	1–2 hrs.	2–4 hrs.	0–1 hr.	1–2 hrs.	2–4 hrs.	0–1 hr.	0–2 hrs.	0–4 hrs.
1	36	F	? Thyrotoxicosis	6.6	35.2	88.4	13.0	8.5	10.9	5.1	8.9	6.1	0.58	1.2	1.8
2	57	F	Myocardial infarct.		35.2	94.1	8.6	5.5	7.5	6.7	3.1	0.6	0.54	0.70	0.73
3	26	F	Lymphoma, Hodgkin's type		74.8	96.5	8.4	17.9	11.1	7.7	7.3	1.3	0.62	1.8	1.9
4	69	F	Congestive heart failure	7.9	74.8	95.0	5.4	4.6		11.4	10.3		0.58	1.0	
5	26	F	Rheumatic heart disease		35.2	85.9		18.4†	12.7		3.6†	0.9		0.57	0.67
6	43	F	Pneumonia; ? tuberculosis		41.2	94.4	14.6	8.7	9.1	6.8	6.8	0	0.94	1.5	1.5
7	47	F	Toxic nodular goiter	9.6	31.7	92.4	8.6	7.5	5.9	7.9	5.0	2.8	0.62	0.96	1.1
8	44	M	Cretin with goiter		36.5	83.1	9.7	7.1	8.1	6.6	4.5	1.4	0.65	0.97	1.1
9	56	M	Myxedema following I-131 therapy; congestive heart failure	2.2	62.2	83.3	1.1	0.9	1.1	25.6	21.0	21.6	0.29	0.44	0.63
10	23	F	Incomplete myxedema	2.0	25.0	70.0	15.1	20.0	21.6	9.1	3.6	6.0	0.94	1.4	2.3
11	67	F	Myxedema	0.9	41.2	94.1	7.9	12.0	3.1	28.4	19.1	18.1	2.1	4.3	4.8
12	52	F	Myxedema after I-131 for thyrotoxicosis	(BMR −34%)	35.2	86.7	6.2	5.8	11.4	22.3	15.8	8.4	1.2	2.0	2.8
13	31	F	Hypothyroid	1.8	40.0	83.1	18.9	8.8	10.4	28.6	17.8	9.9	4.5	5.8	6.6

paper strips were cut at 1-cm. intervals and measured for one minute each in a well-type scintillation crystal detector with a sensitivity of approximately 4×10^5 counts per minute per μc., and a background of 20. Cuttings corresponding to zones of interest on the stained paper strips were counted for two minutes. Quantities of urine were applied to the paper (Whatman #3) which would yield between 2 and 10,000 counts per minute. Usually whole urine was applied. Occasional specimens were so dilute that 2-ml. volumes were acidified with H_2SO_4 and extracted repeatedly with butanol which was in equilibrium with 5 per cent $Na_2S_2O_3$.

RESULTS

The findings are listed in Table 1. Thirteen patients were studied; 5 of them were hypothyroid (Nos. 9–13). One of these 5 (No. 9) had been made myxedematous in an effort to control congestive heart failure by administration of I^{131} six years previously; he had mild congestive heart failure at the time of the study, and also renal failure (serum nonprotein nitrogen level, 58 mg. per 100 ml.) and mild diabetes mellitus. In another patient (No. 12), hypothyroidism developed after I^{131} therapy for Graves' disease. Two (Nos. 10 and 13) were clinically hypothyroid but without the full clinical picture of myxedema. One (No. 11) had classic Gull's disease. Among the 8 "control" patients (Nos. 1–8), 1 had been treated previously by subtotal thyroidectomy for Graves' disease, and 1 (previously reported

by Lerman, Jones and Calkins (5) as a goitrous cretin) had undergone a thyroidectomy ten years previously and was receiving desiccated thyroid.

The fraction of labeled iodine which was present as diiodotyrosine in the urine was distinctly higher in 4 of the 5 hypothyroid patients than in any of the "control" subjects in all collection periods. The fractions excreted as diiodotyrosine by 1 patient with incomplete myxedema were comparable to those of the control group.

The fraction of the administered dose of diiodotyrosine which was excreted unchanged was higher in the hypothyroid group, except for the patient with nitrogen retention. Although in this patient a large fraction of the labeled iodine in the urine was present as diiodotyrosine, the total renal disposal both of iodide and of diiodotyrosine was much lower than in any other patient.

The mean excretion rate of unchanged diiodotyrosine was lower in all three collection periods in the 8 "control" subjects receiving *l*-diiodotyrosine than in 15 patients previously reported who were given labeled racemic diiodotyrosine (2). The differences in each collection period were not striking, and only in the first hour were they significant ($P < 0.02$).

COMMENT

Patients with hypothyroidism appear to be unable to deiodinate *l*-diiodotyrosine as readily as do control subjects, or control subjects given *dl*-diiodotyrosine. This is reflected not only in the finding of a larger fraction of labeled iodine in the urine in the form of diiodotyrosine, but also in the excretion of a larger fraction of the administered dose of diiodotyrosine in an unchanged form.

The rate of metabolism of diiodotyrosine as observed in this group of control subjects who were given *l*-diiodotyrosine was not very different from the metabolism of racemic diiodotyrosine as observed in another group of control patients. Differences in race, age and sex might have accounted for the slightly lower rate of excretion of *l*-diiodotyrosine in the present series, or the differences might have arisen from a slower rate of metabolism of *d*-diiodotyrosine by the patients of the former series. It is apparent, however, that the levo- and the dextro-optical isomers of diiodotyrosine are metabolized at similar rates. This is surprising, in view of the usually much faster rate of metabolism of levo-isomers of amino acids; and especially so, in view of the distinctly different rates of metabolism of dextro- and levo-monoiodotyrosine (2).

SUMMARY

1. The urinary excretion of *l*-diiodotyrosine has been observed in 5 hypothyroid subjects and in 8 patients with other conditions.

2. The patients with hypothyroidism excreted unchanged a larger fraction of the administered *l*-diiodotyrosine than did the control subjects.

3. The rate at which labeled *l*-diiodotyrosine appeared in the urine after administration was slightly less than after administration of labeled *dl*-diiodotyrosine.

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BIOLOGICAL ACTIVITY OF CERTAIN PROGESTERONE-LIKE COMPOUNDS AS DETERMINED BY THE HOOKER-FORBES BIOASSAY*

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PROGESTERONE is unique among steroids in that it is the only progestational hormone characterized from biological sources. There is reason to believe, however, that other substance(s) with this type of activity may be present (1). Bioassays of peripheral blood of pregnant ewes (2) and human beings (3) by the method of Hooker and Forbes indicate levels of as much as 10 μg. per ml. and 2 μg. per ml., respectively. However, chemical extraction of peripheral blood for progesterone resulted in levels which were not measurable (less than 0.1 μg. per ml. of plasma) in the ewe (4), and an average of 0.08 μg. per ml. of blood in the human being (1, 5). By isotopic dilution it was shown that pooled human pregnancy plasma obtained during the second and third trimesters contained 0.08 μg. per ml. of plasma (1). From these data it would appear that the presence of another substance active in the Hooker-Forbes bioassay must be suspected.

During recent years several congeners of progesterone[1] have been prepared and a limited group of these, such as 19-nor-progesterone (6, 8), 11-dehydro-progesterone (9) and 17-α-methylprogesterone (10) have been shown to be equal to, or more potent than progesterone by bioassay in the rabbit or guinea pig. A comparative study in the mouse seemed pertinent

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[1] For the sake of simplicity, the structures of compounds cited in this publication are named as modifications of progesterone, thereby emphasizing the molecular variations tested. For further elucidation, the molecular structures are drawn in Figure 1. We have also tried to avoid the term "derivative" of progesterone to avoid confusion with the more technical "derivative" such as progesterone-hydrazide.

to us for a better understanding of these compounds, since this would furnish a picture of the relative activity of these compounds as well as further definition of the specificity of the Hooker-Forbes test. The Hooker-Forbes bioassay is well suited theoretically for a study of the relationship between structure and activity because of the intimate hormone-tissue relationship which eliminates the intervention of other organs such as the liver.

MATERIALS AND METHODS

The steroids were dissolved in sesame oil and tested on Rockland Swiss mice for progesterone-like activity by the Hooker-Forbes technique (11). A mixture of propylene glycol and 10 per cent albumin (12) was used to dissolve the 17-α-ethynyltestosterone.

RESULTS AND DISCUSSION

The progestational activity of 17 compounds was tested by the Hooker-Forbes bioassay (Table 1, Fig. 1). In almost all cases, the doses were modified until both positive and negative responses were obtained, and therefore some activity was noted for practically all of the compounds tested. For convenience these compounds are arbitrarily divided according to structural modifications.

A. *Removal of a methyl group or hydrogen atoms*

In this study, progesterone is arbitrarily given a potency of 1, indicating a positive reaction at a concentration of 0.3 μg. per ml. and a negative reaction at lower levels. When the angular C_{19} methyl at the C_{10} bridgehead was removed (19-nor-progesterone, (II)), the molecule was slightly less active than progesterone. High activity for this compound has been reported in other species (10) and the difference in potency may reflect no more than species differences.

Removal of two hydrogen atoms from the 11 and 12 carbons (11-dehydroprogesterone, (III)) had little effect on the potency of the compound. Forbes (14) reported that "11-dehydroprogesterone" and "Δ^{11}-progesterone" were less than 1/4 and 1/3000 as active as progesterone respectively. These observations do not agree with our data and those of Klein and Ober (15) who also found that progesterone and 11-dehydroprogesterone were approximately equivalent in activity in the Hooker-Forbes test. On the other hand, altering the B ring (as in 6-dehydroprogesterone (IV)) has a marked effect in lowering the biological activity to 1/60 of that of progesterone. It would appear that the progestational activity of the molecule is particularly sensitive to changes at the alpha-beta unsaturated ketone in the A ring.

TABLE 1. THE BIOLOGICAL ACTIVITY OF CERTAIN STEROIDS IN THE HOOKER-FORBES MICROBIOASSAY

Number	Substance*	Concentration (amt. tested per ml.)	Reaction†	Ratio of activity (progest. test epd.)	Minimum conc. for positive reaction (μg./ml.)
I	Progesterone	1.0 μg. 0.3 μg. 1.0 μg.	++++ +++ −−−−		0.3
II	19-Nor-progesterone	1.0 μg. 0.3 μg. 0.1 μg.	++++ −−− −−−	0.3	1.0
III	11-Dehydroprogesterone	1.0 μg. 0.3 μg. 0.1 μg.	++ +++− −−−	1.0	0.3
IV	6-Dehydroprogesterone	1.0 mg. 0.5 mg. 0.25 mg. 0.10 mg. 0.05 mg. 0.02 mg. 0.01 mg. 0.001 mg.	++ ++ ++ +++ ++ ++ −−− −−−	0.015	20.0
V	17-α-Hydroxyprogesterone	1.0 μg. 0.1 μg. 0.05 μg. 0.03 μg. 0.01 μg. 0.005 μg. 0.003 μg.	+ + ++++ ++++++− +++++ +++ −−−−	60.0	0.005
VI	17-β-Hydroxyprogesterone acetate	10.0 μg. 2.0 μg. 1.0 μg.	++ +++ ++	0.3	1.0
VII	11-Ketoprogesterone	0.1 mg. 0.05 mg. 0.02 mg. 0.01 mg.	++ ++ −−− −−	0.006	50.0
VIII	11-β-Hydroxyprogesterone	0.1 mg. 0.05 mg. 0.02 mg. 0.01 mg. 0.005 mg.	++ ++− ++ −−− −	0.015	20.0
IX	6-β-Hydroxyprogesterone	1.0 mg. 0.5 mg. 0.25 mg. 0.10 mg.	++ −− −− −−	0.0003	1,000.0
X	6β,11-α-Dihydroxyprogesterone	0.15 mg. 0.10 mg.	−− −−	0.002	150.0
XI	11α,17-α-Dihydroxyprogesterone	0.1 mg. 0.05 mg. 0.02 mg. 0.01 mg.	++ −−− −−− −−	0.003	100.0
XII	Pregnane-3,20-dione	5.2 mg. 2.6 mg.	++ −−	0.00006	5,200.0
XIII	5,20-Diketo-3,5-seco-A-norpregnan-3-oic acid‡	1.0 mg.	−−−	0.0003	1,000.0
XIV	5-Hydroxy-20-keto-3,5-seco-A-norpregn-5-en-3-oic-3,5-lactone§	1.0 mg. 0.5 mg. 0.25 mg. 0.10 mg.	+ ++ +++ −−	0.0012	250.0
XV	3-Hydroxy-17-α-dihydroxy,17β-acetyl-1,3,5-estratriene	0.5 mg. 0.25 mg. 0.10 mg. 0.05 mg. 0.02 mg.	+ ++ +++− −− −−	0.0003	1000.0
XVI	3,17α-Dihydroxy-17β-acetyl-1,3,5-estratriene	0.5 mg. 0.25 mg. 0.10 mg. 0.05 mg. 0.020 mg.	++ ++ +++− −− −−	0.003	100.0
XVII	17-α-Ethynyltestosterone	1.0 mg. 0.5 mg. 0.25 mg. 0.15 mg. 0.125 mg.	++ ++ ++ −− −−	0.0012	250.0

* See Figure 1.
† Each + or − represents each mouse tested.
‡ "Keto-acid of progesterone" (13).
§ "Enol-lactone of progesterone" (13).

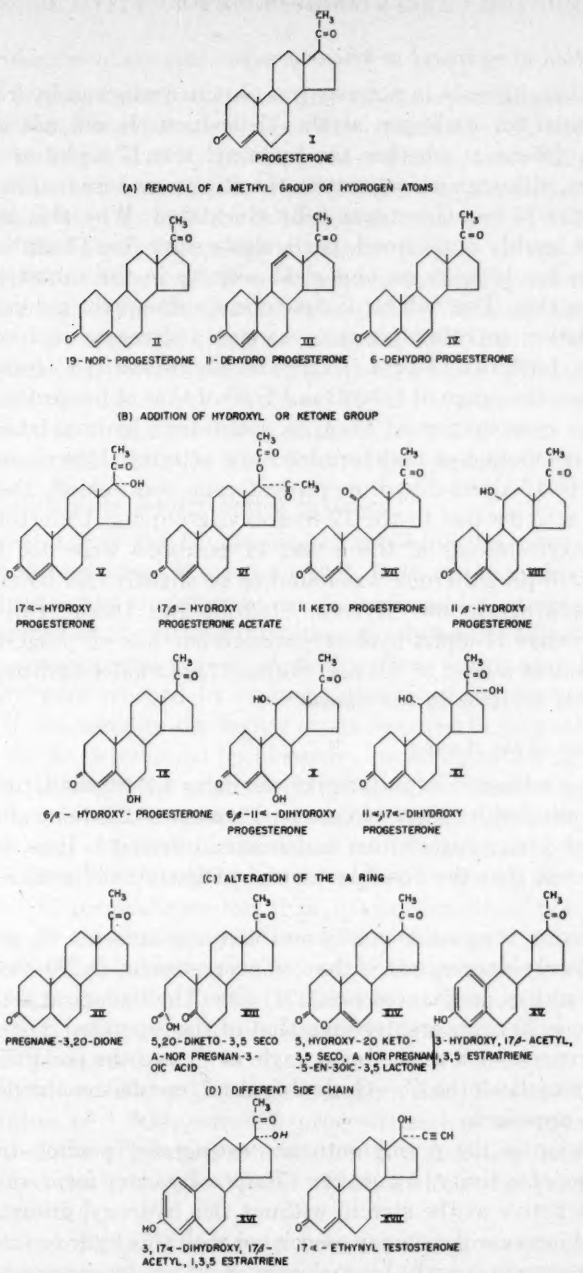

FIG. 1. The molecular structure of a series of steroids resembling progesterone.

B. *Introduction of hydroxyl or ketone groups*

A tremendous increase in potency was obtained when an hydroxyl group was substituted for hydrogen at the 17-position. It did not apparently make much difference whether the hydroxyl was 17-alpha or 17-beta in configuration, although unfortunately the study was terminated before the activity of the 17-beta form was fully elucidated. Why this increase occurred is not readily understood, particularly since free 17-alpha-hydroxyprogesterone has little or no biological activity in the rabbit (16, 18) or human being (18). This will be discussed in a subsequent publication (18).

Hydroxylation in other positions caused a decrease in biological activity. Thus, both the 11-beta (VIII) and the 6-beta (IX) forms resulted in activities in the range of 1/3000 and 1/60 of that of progesterone respectively, and a combination of 11-alpha and 6-beta hydroxylations (X) resulted in a compound of undetermined low activity. It is of interest that when 11-beta-17-alpha-dihydroxyprogesterone was tested, there was an increase in activity due to the 17-hydroxyl grouping. Unfortunately, the alpha-hydroxyl steroids of the 6 and 11 positions were not tested, but singly, 11-keto-progesterone was found to be slightly less potent than 11-beta-hydroxyprogesterone. Byrnes, Stafford and Olsen (19), moreover, have shown that 11-alpha-hydroxyprogesterone has no progestational effect in the rat at a level of 3.0 mg. Forbes (13) found 4-hydroxyprogesterone devoid of activity in the mouse.

C. *Alteration of the A ring*

The well-established importance of the delta 4,3-ketone structure in the A ring was verified by these bioassays. Pregnane-3,20-dione and the keto-acid derived from progesterone had minimal activity. It is, however, of unique interest that the "enol-lactone of progesterone" retained some activity.

Furthermore, the enol-lactone bond distance between O_4 and C_5 (1.42 Å) more closely approximates that of progesterone (1.33) than does the C_4—C_5 distance of pregnanedione (1.54) (20). The biological activity of the enol-lactone is 20 times greater than that of the saturated dione. However, other differences, such as the bond angle at C_4 and the possibility that the C—O link simulates the C_4—C_5 double bond, emphasize the need for caution in this approach.

Modification of the A ring with an "estrogenic" phenol structure also caused a loss of activity. Again, the 17-alpha-hydroxy form was more than 10 times as active as the steroid without this hydroxyl group, confirming the marked increase in potency associated with this hydroxylation. Stereochemical comparison with this molecule could not be expected to be fruitful in the light of the uniqueness of the aromatic ring, not to mention the acidic hydroxyl at the 3 position.

D. *Modification of the side chain*

There is considerable evidence that 11-desoxycorticosterone is a weak progestational hormone when injected systemically (21, 22). However, it has practically no activity in the Hooker-Forbes assay (23). It has been postulated that an intermediate may be formed by the kidney and that this intermediate may account for the progestational activity (24, 25).

Rather than study the many variations possible in the side-chain, 17-α-ethynyltestosterone was chosen because of its high systemic potency and its unusual structure. On direct Hooker-Forbes bioassay the potency was only 1/750 that of progesterone and comparable to that of the enol-lactone. This confirms the observation of Hooker and Forbes (23). It is not possible to interpret this discrepancy, unless it be on the basis of the specificity of the bioassay or on the possibility that ethynyltestosterone is transformed to a more potent substance when injected systemically.

E. *Reliability of the Hooker-Forbes bioassay*

The reliability of the Hooker-Forbes procedure has been questioned in the assay of physiological solutions (26). Both of the authors have shown independently that progesterone can be inhibited by estrogen (26, 27). We have disagreed on the ratio of inhibition, the difference being 1:20 or 1:200 (estradiol:progesterone ratios). Actually, these ratios may be closer, because they were reached by testing serial dilutions, increasing logarithmically. If one assumes the higher levels reported in pregnancy as a basis for analysis (as determined by bioassay), as much as 2.0 μg. per ml. may be present, although the more usual finding is less than 1.0 μg. per ml. (3). On the other hand, the careful analysis of Velduis (28) indicates the presence of 0.03 μg. of estradiol-estrone per ml. of plasma. These ratios, therefore, are 1:70 and 1:35. Although the higher level might be detected reliably, levels of progesterone less than 1.0 μg. per ml. of plasma would be susceptible to the inhibitory effects of the estrogens. Furthermore, the available chemical evidence on the level of free progesterone in blood indicates that it is probably closer to 0.1 μg. per ml. It should be remembered, however, that other progestationally active substances may not demonstrate this phenomenon.

Ober, Klein and Weber (29) have found another effect of estrogen. When optimal ratios of 1:500 (estradiol:progesterone) or less were tested, the sensitivity of the progesterone assay could be increased as much as fivefold. Without doubt these levels fall within the known physiological ranges of both steroids.

The present study introduces a new complication. Steroids known to have greater activity in other species (*viz*, 17-α-ethynyltestosterone, 19-nor-progesterone and 11-dehydroprogesterone) had equivalent or less activity in the mouse intrauterine test. On the other hand, 17-α-hydroxypro-

gesterone, devoid of activity in the rabbit, guinea pig and human, was more than 60 times as potent in this test. It should be emphasized, however, that an advantage of this bioassay is that direct application of the hormone to the tissue eliminates the intermediate effect of other organs modifying the hormone, as might be the case with 17-α-ethynyltestosterone. This does not apply to 17-α-hydroxyprogesterone though, because we obtained a similar effect when it was injected systemically. It might be worthwhile to re-emphasize species differences in the light of the work of Robson and Sharaf (30), who reported a failure to obtain progestational activity in the cat with certain steroids which were active in the rabbit. The special case of 17-α-hydroxyprogesterone will be discussed further in another publication (18).

SUMMARY

Seventeen congeners of progesterone were tested by the Hooker-Forbes intrauterine bioassay. Compounds showing a high degree of activity were 19-nor-progesterone, 17-β-hydroxyprogesterone acetate and 17-α-hydroxyprogesterone. The latter substance was 60 times more active than progesterone. Furthermore, hydroxylation at the 17-position increased biological activity in each steroid which was tested. It was concluded that the discrepancy in results obtained with 17-hydroxylated compounds was not due to the route of administration but might be due to species difference.

The necessity for the intact α-β unsaturated ketone on the A ring was confirmed.

Addendum

Compounds of the pregnen-3-ol-20-one and pregnen-20-ol-3-one structure were not studied extensively in this work. However, it has been recently shown by Zander and his colleagues that pregnen-20α-ol-3-one is a third as active as progesterone by both the Hooker-Forbes and Clauberg tests and that the 20-beta form is twice as active by the Hooker-Forbes test and a fifth as active by the Clauberg test ((a) Zander, J., and Munstermann, A.: *Klin. Wchnschr.* **32**: 894, 1954. (b) Zander, J.; Forbes, T. R.; Neher, R., and Desaulles, P.: *Ibid.* **35**: 143, 1957). After isolation of the alpha form from pregnancy plasma and placenta, pregnen-20α-3-one was found to have the same activity as progesterone ((a) Hooker-Forbes test (1). (b) Salhanick, H. A.; Berliner, D. L.; Jones, J. E., and Holmstrom, E. G., in preparation). As regards the activity of the 3-ol forms, Butenandt and Schmidt (*Berl. deutsch. chem. Gesellsch.* **67**: 2092, 1934) state that Hohlweg indicated that 20 mg. had no noticeable effect on the mucosa of the infantile rabbit, whereas progesterone was effective at dosages of 0.5 to 0.75 mg.

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Basle, Switzerland. In addition to providing us with the enol-lactone of progesterone, Dr. George Fujimoto of Albert Einstein College of Medicine was consulted on many chemical problems.

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BIOLOGICAL ACTIVITY OF 17-α-HYDROXYPROGESTERONE IN THE MOUSE, RABBIT AND HUMAN BEING*

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INTRODUCTION

IT WAS reported in a previous publication (1) that 17-α-hydroxyprogesterone is 60 times more potent than progesterone as measured by the Hooker-Forbes bioassay in the mouse. In the rabbit, however, Junkmann found that the free compound was inactive (2). As there appeared to be a species difference, and as the addition of the hydroxyl group to the 17 position of other steroids of low potency increases their activity, it was of interest to extend this study to include the rabbit and the human being. The progestational reaction in the rabbit has been accepted as the biological criterion for this class of compounds since progesterone was first isolated, and use in human therapy is one of the most practical goals in the study of progestational compounds.

Recently, 17-α-hydroxyprogesterone and its caproate ester have gained attention by clinical use. Junkmann (2) found that the caproate ester of 17-α-hydroxyprogesterone, but not the free compound, had prolonged action in the rabbit. Davis and Wied (3) tested only the esterified steroid in the human being and reported a more prolonged action than that obtained with progesterone.

We are presenting our data comparing the activity of the free compound (17-α-hydroxyprogesterone) in the mouse, the rabbit and the human subject.

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MATERIAL AND METHODS

Both progesterone and 17-α-hydroxyprogesterone were dissolved in sesame oil for injection. When larger doses were indicated, as was the case in the human subjects, a sterile suspension in sesame oil was used. For oral administration the crystals were given in a capsule. The Hooker-Forbes intrauterine test was performed on Rockland Swiss mice according to the standard procedure (4). For testing in the rabbit, either the Corner-Allen bioassay or the McGinty test was used (5, 6). Human studies were carried out on women with endometrial hyperplasia, and the effect of the steroid was determined by endometrial biopsy both before and after the administration of the steroid.

RESULTS

A. *Potency in the mouse*

These results are more elaborately discussed in a prior publication (1). It may be seen from Table 1 that 17-α-hydroxyprogesterone was approximately 60 times more active than progesterone, as 2×10^{-4} μg. of progesterone caused positive responses in all 3 of the animals tested whereas similar activity was elicited by only 3×10^{-6} μg. of 17-α-hydroxyprogesterone.

B. *Potency in the rabbit*

The usual Corner-Allen gradation of activity was observed for progesterone and indicated a sensitivity of the same order as that reported by others. However, as much as 50 mg. of 17-α-hydroxyprogesterone injected intramuscularly had no effect, even when the steroid was administered directly into the endometrium in doses of 2.5 to 5.0 mg.

C. *Progestational activity in the human subject*

Case 1. E.D. (#92-95). This woman was 32 years old. Endometrial biopsy prior to the intramuscular injection of 50 mg. of 17-α-hydroxy-progesterone showed endometrial hyperplasia, indicating that estrogenic priming had been adequate (Fig. 1A). Biopsy was repeated seven days after the administration of 17-α-hydroxyprogesterone, but there was no evidence of a progestational effect (Fig. 1B). A dose of 50 mg. of progesterone was then administered intramuscularly. Four days later withdrawal bleeding occurred.[1] Endometrial biopsy at the onset of this bleeding showed a well-defined progestational effect in the stroma cells (Fig. 1C).

Case 2. S.H. (#6-00-80). This woman was 20 years old. Endometrial biopsy three days prior to the administration of 50 mg. of 17-α-hydroxyprogesterone showed a well developed endogenous estrogenic effect in the endometrium (Fig. 2A). Withdrawal bleeding did not occur in the subsequent eight days. Biopsy was repeated. There was no evidence of progestational effect, but the effect of endogenous estrogen was more pronounced. A dose of 50 mg. of progesterone was administered intramuscularly on the same day. Four days later, there was withdrawal bleeding.

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[1] The administration of 50 mg. of progesterone in the human will always provoke withdrawal bleeding four days later if the estrogen priming of the endometrium has been adequate and ovulation has not occurred.

TABLE 1. COMPARISON OF PROGESTERONE AND 17-α-HYDROXYPROGESTERONE IN MOUSE, RABBIT AND HUMAN

Substance	Species	Dose	Route	Activity
Progesterone	Mouse*	2×10^{-4} μg.	i.u.†	3/3 positive responses.
		1×10^{-4} μg.	i.u.	0/4 positive responses.
17-α-Hydroxy-progesterone	Mouse	6×10^{-6} μg.	i.u.	5/5 positive responses.
		3×10^{-6} μg.	i.u.	3/3 positive responses.
		1.5×10^{-6} μg.	i.u.	0/4 positive responses.
Progesterone	Rabbit‡	0.5 mg.	i.m.§	One-plus reaction.
		1.0 mg.	i.m.	Two-plus reaction.
		1.5 mg.	i.m.	Three-plus reaction.
17-α-Hydroxy-progesterone	Rabbit	10.0–50.0 mg.	i.m.	Inactive.
		2.5– 5.0 mg.	i.u.	Inactive.
Progesterone	Human‖	25.0 mg.	i.m.	Progestational stroma effect and withdrawal bleeding.
		50.0 mg.	i.m.	Progestational stroma effect and withdrawal bleeding.
17-α-Hydroxy-progesterone	Human	50.0 mg.	i.m.	No effect.
		100.0 mg.	i.m.	No effect.
		100.0 mg.	oral	No effect.

* Hooker-Forbes intrauterine bioassay.
† Intrauterine injection.
‡ Corner-Allen bioassay (intramuscular).
§ Intramuscular injection.
‖ Endometrial biopsy.

Case 3. M.L. (#2-97-50). This woman was 40 years old. Endometrial biopsy was performed on the same day on which a dose of 50 mg. of 17-α-hydroxyprogesterone was injected intramuscularly; the endometrium was hyperplastic. A second biopsy performed six days later showed no signs of progestational activity. Withdrawal bleeding did not occur.

Case 4. J.H. (#1-89-22). This woman was 48 years old. On the day she was given 100 mg. of 17-α-hydroxyprogesterone orally, biopsy showed that the endometrium was hyperplastic. No withdrawal bleeding ensued. However, four days after the administration of 50 mg. of progesterone intramuscularly, withdrawal bleeding occurred.

DISCUSSION

These observations confirm the importance of species differences in the action of progestational substances. Whereas results with the Hooker-

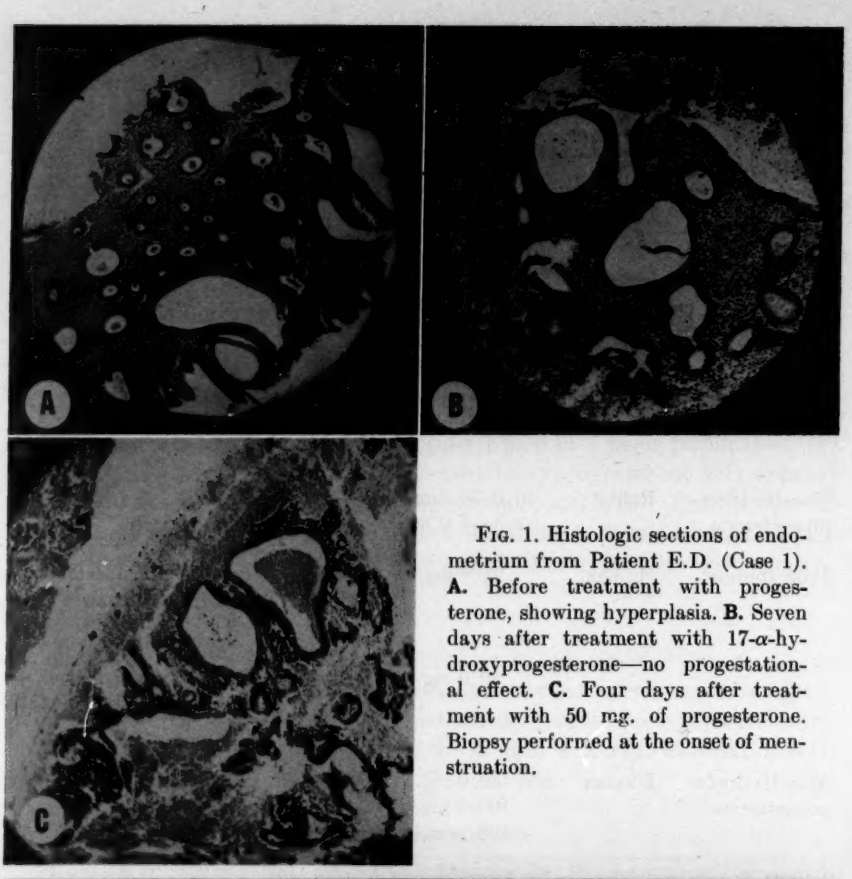

FIG. 1. Histologic sections of endometrium from Patient E.D. (Case 1). **A.** Before treatment with progesterone, showing hyperplasia. **B.** Seven days after treatment with 17-α-hydroxyprogesterone—no progestational effect. **C.** Four days after treatment with 50 mg. of progesterone. Biopsy performed at the onset of menstruation.

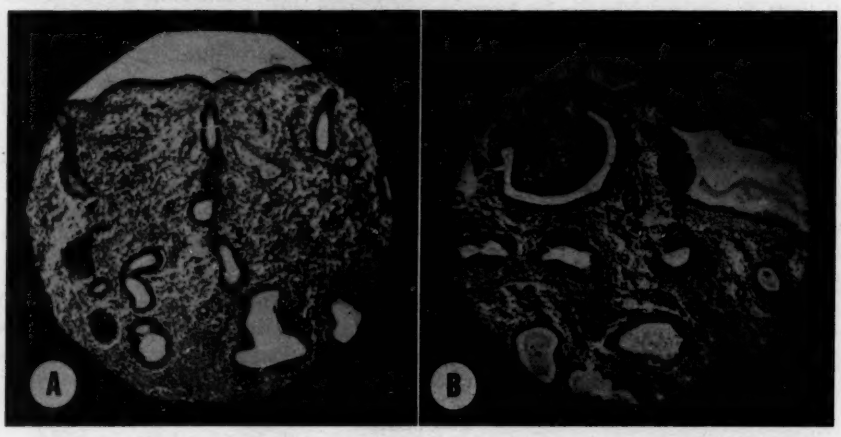

FIG. 2. Histologic sections of endometrium from Patient S.H. (Case 2). **A.** Before treatment, showing a good proliferation. **B.** One week after the administration of 50 mg. of 17-α-hydroxyprogesterone—no progestational effect.

Forbes assay in the mouse indicated a 60-fold superiority for 17-α-hydroxyprogesterone as compared with standard progesterone, the compound was inactive in the rabbit and in the human being. It is perhaps significant that the uterus of the rat under physiological conditions does not develop a progestational endometrium comparable to that seen in the rabbit (7), and that the rat and the mouse appear to be similar in this respect (8). In addition, it has been shown that even under the influence of progesterone the endometrial changes in the uterus of the mouse are not comparable to the typical histological pattern seen in the rabbit or human being (8, 9).

The patterns in the rabbit and human being are similar, and therefore pertinent to the problem of measuring progestational activity. As regards 17-α-hydroxyprogesterone, our observations confirm those of Junkmann (2) and Pfiffner *et al.* (10). 17-α-Hydroxyprogesterone is, at most, one quarter as active as progesterone, and probably much less. Oral administration does not increase the activity, as might be expected if it were transformed into a more potent substance.

The biological difference between the free alcohol and the caproate ester is difficult to explain. Several workers have found an increase in activity with the caproate ester (2, 11, 12). A point of significance may be the different processes or rates of degradation which the two forms undergo (13). Thus, Langecker (13) found in the male that the free alcohol of 17-α-hydroxyprogesterone was excreted as pregnane-3,17,20-triol and pregnane-3,17-diol-20-one but found no change in the excretion of pregnane-3,20-diol or 17-ketosteroids. On the other hand, the caproate ester administered to a man could not be recovered from the urine in amounts greater than 1 per cent. Administration of the caproate to a pregnant woman resulted in an increased excretion of pregnane-3,20-diol.

It does not seem reasonable that the caproate ester elicits increased progesterone secretion from the ovary, because Davis and Wied (3) found that it had high potency in castrated women. It is possible that absorption is better from the dissolved solution of the caproate than it is from the suspension of the free alcohol in sesame oil, but if this be the case, one would anticipate an excretion pattern the opposite of that in Langecker's study (13). It would not be anticipated that scission of the caproate occurs at the carbon 17-caproic acid bond (yielding caproic acid and free progesterone as a precursor to pregnanediol) without the assistance of an unusual enzyme system. Thus the mechanism of action of these substances remains obscure.

SUMMARY

The free alcohol of 17-α-hydroxyprogesterone is shown to have little or no progestational activity in the rabbit or human being, although it is

approximately 60 times more active than progesterone in the mouse. This is contrary to the known activity of the caproate ester of 17-α-hydroxyprogesterone, but confirms published reports on its activity in the rabbit. No explanation can be offered at this time for these observations.

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AN IMPROVED ALL-GLASS EXTRACTOR

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A VERSATILE automatic extractor suitable for removing steroids from urine was devised by Cohen (1) in 1950. The driving force was a mild vacuum (house vacuum or water aspirator) which induced an alternate pressure decrease in the upper chamber, causing urine to be drawn up the delivery tube, to drop through the extractor tube, and to percolate in the form of drops through a comparatively large volume of the lower solvent phase. The extraction was shown to be effective at room temperature; and, under conditions of equilibrium, 90 per cent or more of the total extractable steroids could be found in the solvent phase (chloroform or carbon tetrachloride). The time required depended upon the volume of urine and the rate at which the extraction proceeded.

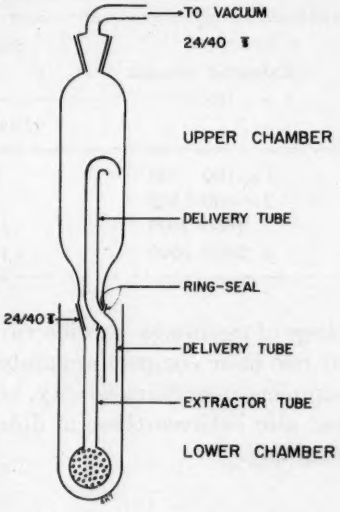

FIGURE 1

Described in this paper is an all-glass automatic extractor[1] that retains Cohen's principle of operation, but which is simpler and easier to employ, and has a greater range of utility (Fig. 1). It consists of four parts: (a) a connection to the source of vacuum, (b) an upper chamber into which the upper half of the delivery tube is ring-sealed, (c) a unit containing the lower half of the delivery tube and extractor tube, and (d) a lower chamber containing this unit. The apparatus may be used at room temperature with various solvents either heavier or lighter than water, such as chloroform and carbon tetrachloride, or ethyl acetate and n-butanol, respectively. The changes in design from Cohen's original apparatus allow the upper chamber to be surrounded with a constant temperature bath, thus permitting beta-glucuronidase hydrolysis of urinary

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[1] Lanning Glass Co., Vineland, N. J.

steroid conjugates in the upper chamber at 37° C. while chloroform extraction of the released steroids is proceeding simultaneously in the lower chamber at room temperature. The extractor may also be employed for the simultaneous extraction and dialysis of steroids from blood (2) or tissue homogenates (3) in place of the larger and more expensive one described by Wolfe and Hershberg (4). Four sizes of extractors have been designed (Table 1) for varying volumes, all of which remove approximately 90 per cent of the extractable steroids at equilibrium.

The advantages of this apparatus over those of Cohen and Wolfe-Hershberg are: 1) the absence of all rubber stoppers, which eliminates a possible source of contamination to both urine and solvent; 2) an increased

TABLE 1. THE FOUR SIZES OF EXTRACTOR CHAMBERS (UPPER AND LOWER) AND THE RANGE OF VOLUMES MOST APPLICABLE TO THEIR USE

Extractor volume (ml.)	Size of upper and lower chambers (cm.)		Sizes of joints
	Diameter	Length	
1. 100– 500	5	30	24/40
2. 600–1500	7.5	40	24/40
3. 1600–2500	10	50	29/42
4. 2800–5000	10	80	29/42

range of usefulness—a wide variety of extraction problems may be handled by one basic compact apparatus; 3) standard tapered joints which permit complete interchangeability, not only between extractors of the same size but also between those of different sizes, and 4) ease of cleaning and assembling.

SUMMARY

An improved all-glass automatic extractor is described which is based on the principle of Cohen but which may be used for a wider variety of extraction problems than either the Cohen extractor or the conventional Wolfe-Hershberg type. It is useful in the extraction of steroids from urine, blood and tissue homogenates with solvents either heavier or lighter than water, and operates at room temperature.

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Editorial Review

DEVELOPMENTAL ASPECTS OF HUMAN HERMAPHRODISM: THE SIGNIFICANCE OF SEX CHROMATIN PATTERNS

FROM several independent sources of scientific research, new light has been focused on the problem of human intersexuality. Of paramount significance have been studies in experimental embryology and sex-specific chromatin patterns which can be identified in the inter-mitotic nuclei of some mammals.

EXPERIMENTAL EMBRYOLOGY

It has been demonstrated that in lower vertebrates differentiation of the embryonic gonad is not irrevocably pre-fixed by genetic constitution, but can be affected by external factors. Testes may very well develop in genetic females, if the proper hormonal or physical influence is exerted while the embryonic gonad is still in the indifferent stage. Conversely, ovaries may be induced to differentiate in genetic males. Since this work has already been reviewed (1), it is only necessary here to re-emphasize important principles. A striking example is the creation of an all-male colony of Xenopus, a South African frog, by Chang and Witschi (2). In this species the male is homogametic. A normal homogametic male was mated with a genetic male which had been sex-reversed into a reproducing female, physically indistinguishable from a normal heterogametic female. All offspring from this mating were homogametic, and therefore, all male, since both parents were homogametic. From this demonstration, two basic principles can be formulated: 1) the embryonic gonad and secondary sex apparatus has the ability to differentiate oppositely to the genetic sex constitution; and 2) in spite of genetic constitution, germ cells retain a bipotentiality, *i.e.*, genetically male germ cells can differentiate as ovogonia, undergo ovogenesis, and produce functional ova.

Other work in experimental embryology has advanced our understanding of embryogenesis of the secondary sex organs, so that in this area also certain basic principles which relate to intersexuality have been established. It is generally accepted that differentiation of most secondary characters is not under direct genetic control, but rather is under the influence of secretions from the embryonic gonad, as shown by Jost's experiments on intra-uterine castration of rabbit fetuses (3). This principle was con-

firmed by Jost and Bergerard (4) and Price (5) by culturing embryonic sex primordia of rats *in vitro*. They demonstrated that in the absence of either ovaries or testes, there is regression of the mesonephric (Wolffian) duct, and female secondary sex structures (Müllerian duct derivatives and external genitalia) develop. These findings are particularly significant, because in the experiments the gonadal influence was eliminated without risk of damaging neighboring primordia. They indicate that in rats and rabbits, at least, the female condition is neutral with regard to development of secondary sex ducts and external genitalia, whereas male-type development requires the inductive influence of fetal testes. Not all secondary sex characters, however, are controlled by gonadal influences. Examples of exceptional features which differentiate according to genetic sex, regardless of the functional sex, have been described for the opposum (6), birds (7) and selachians (8). These characters, controlled directly by genetic constitution, can be used as true "markers" of chromosomal sex, even though experimental interventions may have induced partial or even complete sex reversal. Thus, among the structural features that show sexual dimorphism, some follow the lead of the gonads by assuming either male or female characters, whereas others follow directly and inalterably the genetic sex as determined at the time of zygote formation.

NUCLEAR CHROMATIN PATTERNS

In the course of studying the nuclear morphology of neural cells in the cat, Barr and his associates discovered a previously ignored sexual dimorphism that permits the identification of male or female tissue (9, 10). Extending this study to several mammals, including man, it was possible to identify female tissue by the presence of a characteristic chromatin mass, which is absent or only occasionally seen in male cells. Human skin (11, 12), leukocytes (13), scrapings from the buccal mucosa (14, 15), and vaginal exfoliate cells (16) all provided readily available sources of material for the identification of this sex-specific characteristic. Typical results are exemplified as follows:

	Number of cases	*Percentage of nuclei with chromatin masses*
Skin biopsies (normal males)	90	4.7 (range, 1–12)
Buccal smears (normal males)	146	0.7 (range, 0–4)
Skin biopsies (normal females)	74	69.0 (range, 51–82)
Buccal smears (normal females)	93	53.0 (range, 20–79)

It has been proposed by Barr (17), and the interpretation adopted by several others (18, 19, 20), that the characteristic planoconvex chromatin

mass may represent a fused heterochromatic region of the XX sex chromosomes. The XY sex chromosome pair of males presumably does not result in duplication of this heterochromatic locus, so that a comparable structure is not visible. On this basis, it would become justifiable to relate nuclear morphology to genetic sex. At the present time, however, this important correlation is on theoretical grounds only. As Barr (21) has pointed out, although the chromatin mass-sex chromosome relationship seems the most acceptable explanation for these observations, it should be adopted with the realization that convincing proof has not been demonstrated.

In this regard it may be worthwhile to recall such observed facts as: 1) a characteristic heteropyknotic body can be seen in over 50 per cent of the nuclei of normal female cells; 2) a similar heteropyknotic body is seen in less than 5 per cent of normal male cells; 3) this mass reacts in a strongly positive manner with the Feulgen stain and thus can be regarded as heterochromatic material; 4) the chromatin mass can be identified with its characteristic sex distribution in the embryo; and 5) the chromatin mass is not influenced by exogenous hormone administration, nor by an abnormal endogenous hormonal status. Thus, in the cell nuclei of individuals who are normal males on the bases of gonads and bodily habitus, there is invariably a relative absence of chromatin masses. Furthermore, tissue from normal females invariably can be recognized as such by the high frequency of nuclei in which the chromatin mass can be demonstrated. However, it has not been directly observed that the heterochromatic mass is related to the sex chromosomes, nor is it certain that the small mass of heterochromatin noted occasionally in male cells is homologous with that seen in female cells. At the present time, geneticists are not in agreement with regard to the significance of inter-mitotic heterochromatin. There is no more than equivocal evidence in favor of the supposition that such a chromatin mass is more likely to be related to the X chromosome than to one or another of the somatic chromosomes (22). One might as reasonably support the theory that the heterochromatic mass represents intermitotic allocyclic regions of an autosomal pair containing male-determining factors. To justify this point of view, it is first necessary to review briefly the quantitative or "balance" theory of sex determination.

Relationship of sex chromatin mass to chromosomal loci

A complete explanation of this modern concept of sex inheritance can be found in the recent book of Richard Goldschmidt (22), but for the purposes of the present discussion, certain principles only need be high-lighted: 1) male and female genetic sex determiners are present in both sexes, accounting for the fact that in each sex the potentiality for the other sex is present; 2) the existence of a quantitative relation or "balance" between

the two types of sex determiners decides sexuality (maleness or femaleness); 3) determiners of one sex type are located within the X-chromosome (female determiners in the human), whereas determiners of the other type occur outside of the X-chromosome; 4) the sex determiners outside of the X-chromosomes (in the case of humans, the male determiners) may occur on the Y-chromosome *or on a pair of autosomes;* and 5) the "balance" system works so that two doses of the female determiners in the X-chromosomes (XX) overcome the male determiners outside the X, but one dose (XY) is insufficient for the dominance of female factors.

Proceeding from this well-documented theory, it is possible that the heterochromatic mass visualized in female nuclei actually represents the autosomal locus carrying the hypostatic (suppressed) male determiners, rather than heterochromatic portions of the X-chromosomes. Each of these explanations, however, fails to account for the puzzling occurrence of the chromatin mass in a small percentage of male cells. We cannot be certain as yet that the mass seen in male nuclei is homologous with that observed in female nuclei, and it is possible that the occasional male nuclei with atypical heterochromatic bodies are nothing more than cells in which chromosomal fragmentation and subsequent heteropyknosis has taken place in the course of mitotic division. Studies of the ultrastructure of male and female chromatin masses with the electron microscope may aid in the elucidation of this puzzling problem.

There is a basic difference between the chromatin mass-sex chromosome theory and the concept that the chromatin mass is related to one or another of the autosomes. In the former, heteropyknosis is related to the epistatic (active) sex determiners, *i.e.*, the female determiners of the X-chromosome, whereas the latter concept suggests that the hypostatic (inactive) male sex determining genes assume the heteropyknotic character. It is noteworthy that Serra (23) concluded that non-function, from a genetic point of view, is characteristic of heterochromatic chromosomal regions as distinguished from actively functioning euchromatic loci.

The suggestion that the chromatin mass may be related to autosomes rather than X-chromosomes is offered not so much for the purpose of replacing one theoretical explanation for another, as it is to emphasize the equivocal nature of the chromatin mass-sex chromosome concept. Can we, therefore, discount the idea that the chromatin mass has a relationship to true genetic sex? Obviously not, for two important reasons: 1) even the alternative explanation would relate the chromatin mass directly to the genetic determination of sex, though by a different mechanism; and 2) the accumulated information on nuclear morphology has given this feature of microscopic sexual dimorphism a role sufficiently significant to warrant recognition as a true sex-specific characteristic. The observation that this

characteristic is unaffected by hormonal status, is unaffected by castration, appears from embryonal stages, and is present until death, would classify it as a secondary sex characteristic that differentiates according to genetic sex without regard to functional sex (as has been noted in examples from studies in experimental embryology). It is reasonable to presume, by analogy with the observations on sex-specific morphologic characters in lower vertebrates (6–8), that the sex-chromatin mass provides us with a genetically determined "marker" of epistatic sex, regardless of the direction taken in apparent sex differentiation.

Application to human intersexuality

When this significant development in the field of nuclear morphology is viewed in conjunction with recent advances in experimental embryology, it is possible to undertake an intelligent re-evaluation of the subject of human intersexuality. The initial application was the observation by Polani, Hunter and Lennox that 3 patients with "ovarian agenesis" all had a male chromatin pattern (24). Other reports followed (25, 26), and it has become apparent that the great majority of these individuals are genetic males. It is proper, therefore, to designate such cases as gonadal agenesis or gonadal dysgenesis.

Gonadal agenesis (dysgenesis). In our series of 117 cases, some of which have been reported previously, the nuclear chromatin data were as follows:

Gonadal agenesis	*Number of cases*	*Percentage of nuclei with chromatin masses*
Cases which were considered "genetic" males on basis of skin biopsy or buccal smear.	98	2.9 (0–14)
Cases regarded as "genetic" females on basis of biopsies or smears.	19	47.5 (25–83)

The ratio of males to females on the basis of nuclear pattern was essentially 5 to 1. The clinical data in each of the 117 cases were sufficient to indicate that we were dealing with examples of gonadal agenesis. As was true for the animal experiments cited earlier, in the absence of gonads the neutral or female condition of the secondary sex characters develops, so that when this syndrome occurs in individuals of genetic maleness, a dichotomy exists between sex determination and secondary sex differentiation. The experiments of Jost and others offer no explanation for the high incidence of genetic males in gonadal agenesis. Other considerations are necessary if an explanation of the embryologic phenomena leading to gonadal agenesis is to be sought. This problem will be re-introduced presently.

Klinefelter's syndrome. A second unexpected finding was the appearance

of the female-type nuclear pattern in cases of the so-called "Klinefelter's syndrome." This observation, reported almost simultaneously by several investigators (27, 28, 29) has been suggested by Witschi as having a relationship to a possible deficiency in primordial germ cells (30). On the basis of amphibian experiments, he concluded that both types of almost complete sex reversal in man, the "pseudo-female" (gonadal agenesis—genetic male) and the "pseudo-male" (Klinefelter's syndrome—genetic female) owe their origin to defects (such as over-ripeness) in the unfertilized egg. According to this concept, such defects result in degeneration of the germ cells during embryogenesis and the formation of sterile, or nearly sterile, gonadal folds immediately preceding sex differentiation. According to Witschi, sterile primordia cause gonadal agenesis and consequent development of the female somatic habitus. Primordia with a reduced number of germ cells become rudimentary testes and direct a male-type differentiation of the somatic sex characters. In each instance, the genetic sex constitution is unable to express itself.

Although there is substantial experimental basis for this theory in the studies of Witschi and his students on amphibia (31), it is problematic whether direct proof will be forthcoming from human material. Particularly important, but lacking at the present time, are observations on the pre-puberal condition of the gonads in "Klinefelter's syndrome," so that we can only assume that the germ cell deficiency dates back to embryonic stages. The basic fact remains in this situation, as in gonadal agenesis, that there is a dichotomy between sex-determination and sex-differentiation and that it arises as a consequence of an undefined embryonic dysgenesis.

The recent re-examination of "Klinefelter's syndrome" has led to a more exact classification of such cases on the basis of testicular morphology and the determination of nuclear sex type. This classification of the syndrome into "true" and "false" types has been described in a recent publication (32) and requires no more than brief summary here. In so-called "true" Klinefelter's syndrome the vast majority of tubules are completely hyalinized and are very small—an indication that the sclerotic process occurred prior to the time that the tubules usually undergo their normal pubertal enlargement. Variable numbers of tubules show little or no hyalinization, and only rarely have residual germinal elements been observed. In "false" Klinefelter's syndrome the testes have undergone post-pubertal sclerosis, usually as a consequence of orchitis of recognized or unrecognized causes. The seminiferous tubules may be completely sclerosed, but they are recognizably larger and more uniformly spaced, as would be anticipated for normal testes, than in the "true" Klinefelter's syndrome. An analysis of nuclear morphology in oral smears or skin biopsy specimens reveals the interesting fact that the histologic classification of "true" Klinefelter's

syndrome appears to be invariably paired with female-type nuclei, and that of the "false" syndrome with male-type nuclei. A summary of findings in 67 cases is presented in the following table. (The diagnosis of "true" or "false" Klinefelter's syndrome was made in each instance before the result of the nuclear counts was known.)

Klinefelter's syndrome	*Number of cases*	*Percentage of nuclei with chromatin masses*
"True"	54	44.6 (22–75)
"False"	13	1.0 (0– 3)

CONSIDERATIONS OF THE CAUSATIVE FACTOR IN SEX REVERSAL

Confining our considerations at this juncture to the "true" syndrome, this condition provides an example of the capacity of the secondary sex apparatus in human subjects to differentiate oppositely to the genetic sex constitution, as in gonadal agenesis. In addition it demonstrates the bipotentiality of the germ cells, *i.e.*, genetically female germ cells differentiate as spermatogonia, undergo spermatogenesis and produce recognizable spermatozoa (Fig. 1). Bunge and Bradbury noticed isolated tubules with spermatogenic activity in cross-sections of testes from 3 cases of "true" Klinefelter's syndrome and came to the conclusion that the presence of male-type gametogenesis is apparently contradictory to the occurrence of female-type chromatin in the somatic cells (33). They write that "by tradition" the presence of spermatogenesis would invalidate the female sex chromatin findings. This opinion is based apparently on the assumption that spermatogenesis can occur only in genetic males. Tradition notwithstanding, this is contrary to the observed facts, and the point should be emphasized for the sake of those readers who are unaware of the findings of experimental embryology. Although the point has already been made, it is important to reiterate that genetic female amphibians can be reversed to sperm-producing, functional males by the administration of hormones at a critical time in development (2). In female chicks, if the functional left ovary is removed, the right gonad, although its complement of germ cells is meager, assumes complete spermatogenic activity (34). The occurrence of ovotestes in mammals also demonstrates the principle that germ cells are bipotential and, depending on extrinsic factors, can take either the spermatogenic or the ovogenic path of differentiation. The recent finding of complete spermatogenesis in the testis of a patient with "true" Klinefelter's syndrome (Fig. 1) illustrates this principle again in a naturally occurring dysgenesis.

Whether the causative factor for almost complete sex-reversal in such cases has its origin: 1) with the unfertilized egg, as suggested by Witschi, 2) at the time of fertilization, or 3) during *in utero* development while gon-

adal differentiation is susceptible to extrinsic interference, remains unanswerable at the present time. Must we not, however, recognize the possibility that a somewhat more "efficient" exertion of this unknown causative factor might complete the process of nature's sex reversal? It is a long step from the type of gonad shown in Figure 1 to fertility; but a few months

FIG. 1. Testicular biopsy in a case of Klinefelter's syndrome, with female chromatin pattern. Note group of tubules exhibiting complete spermatogenic cycle: dividing spermatogonia, spermatocytes, spermatids and spermatozoa. Elsewhere the biopsy showed hyalinized tubules only. (×200.)

ago, the occurrence of complete spermatogenesis in a human genetic female would have been regarded as equally unlikely.

The uncertainty of *primary* causation, in terms of deficiency in the unfertilized egg, genetic factor or *in utero* influence, pertains fully as well in other conditions of human intersexuality. Nevertheless, it is possible to describe the altered events in embryogenesis of the gonads and/or the secondary sexual organs, and also to deduce the time in development when the manifestations of abnormality must have occurred. At $7\frac{1}{2}$ weeks of age, the human embryo is at the sexually indifferent stage, the germ cells have migrated into the gonadal ridge, and the indifferent gonad is now

ready to realize the genetic sexuality which had been determined at fertilization. Genetic maleness will, in the normal course of events, result in dominance of the medulla of the indifferent gonad. As a consequence the germ cells are incorporated into the medullary region, the cortex regresses, and testicular development occurs. On the other hand, genetic femaleness results in dominance of the cortex of the indifferent gonad, retention of germ cells in the cortical cords, dispersion of the medullary tissue, and ovarian development. Embryogenesis of the internal and external secondary sex characters in a cephalo-caudal progression then follows the influence of gonadal differentiation. Organization of the duct system is completed during the remainder of the first half of pregnancy, but the external genitalia continue to differentiate after mid-term. On the basis of this brief sketch of sexual embryogenesis, and in conjunction with the chromatin-pattern technic for ascertaining genetic sex, it is possible to consider the alternative possibilities that might give rise to each of the major forms of human intersexuality.

Congenital adrenal hyperplasia

This condition is pertinent to the present discussion, since its occurrence in females during embryonal life frequently leads to uncertainty regarding true sex at birth. In these cases gonadogenesis follows genetic sex determination, and early differentiation of the secondary sexual structures progresses normally. The nuclear pattern in 59 cases was as follows:

Congenital adrenal hyperplasia	*Number of cases*	*Percentage of nuclei with chromatin masses*
Females	56	53.6 (26–78)
Males	3	2.5 (0– 4)

During the second half of pregnancy, the production of unusual quantities of androgen by the fetal adrenal affects the later phases of secondary sex differentiation, particularly the urogenital sinus, labio-scrotal folds, and the phallus. Female embryos appear to be affected more frequently, although it is probable that this simply reflects the fact that the newborn female who has been under the influence of unusual amounts of androgen is more likely to come under scrutiny. In some instances, the effect upon the female external genitalia is so extensive that the individuals are reared as boys. Laparotomy in these cases reveals the presence of ovaries, oviducts and uteri.

An experimental "adrenogenital" syndrome in frogs has been described (35, 36) in which there occurs, concomitant with adrenal hyperplasia, complete reversal of gonadal differentiation in genetic females. That these two phenomena are independent of each other was subsequently shown (37). It is therefore possible that human cases may eventually appear in which some form of gonadal dysgenesis occurs concurrently with congenital

adrenal hyperplasia. We should anticipate such a finding and be prepared to realize that a cause and effect relationship does not necessarily exist.

If we recognize the adrenal androgens as the direct virilizing factor, is there any clue to the mechanism of their hyperproduction? The pronounced familial distribution indicates a genetic predisposition toward this defect of adrenal function, but the process by which such a genetic factor is translated into physiologic mechanisms is not entirely clear. It would seem probable that the *in utero* milieu is not primarily the source of stimulation of the fetal adrenal, because cases have been recorded in which congenital adrenal hyperplasia occurred in only one of fraternal twins (38). Existing evidence indicates that affected individuals suffer a metabolic defect which results in enhancement of 17-ketosteroid production at the expense of the 11-oxygenated corticoids. Recent findings (39) point to a deficiency of adrenal "21-hydroxylase," an enzyme essential to the synthesis of compound F. The genetic factor accounting for this enzymatic defect is apparently a multiple or quantitative allele which may manifest its presence early in embryonic development, or after a variable period of latency, so that the enzyme deficiency occurs later in life. In the latter instance, there is no influence on the differentiation of the genital tract.

The following table summarizes the embryologic events in congenital adrenal hyperplasia occurring pre-natally:

Congenital Adrenal Hyperplasia

Embryologic event	*Course of embryogenesis*	*Suggested influence*
Gonadogenesis	Normal	——
Early differentiation of secondary sex characters	Normal	——
Late differentiation of secondary sex characters	Virilized	Adrenal androgens. Deficiency in adrenal "21-hydroxylase"

Women with testes (male pseudohermaphrodites)

These individuals are of female appearance at birth. At puberty some masculine features may occasionally develop. The gonads are undescended testes, and usually Wolffian-duct derivatives such as vas deferens and epididymis are present. Imperfect remnants of Müllerian-duct structures and a vagina, either of essentially normal proportions or little more than a urogenital sinus, are also present. The clitoris may be normal until puberty, when enlargement occurs in some instances. Pubic and axillary hair are variable and may be completely absent. In 19 cases of this syndrome on which material has been available, buccal smears, skin biopsy specimens, or both have been examined, and in each instance the nuclear pattern was male (the average percentage of nuclei with chromatin masses was 5.1, with a range of 0 to 10). Although we cannot be sure from this small series that the genetic sex in this syndrome is invariably male, it seems probable

that such is the case. Family histories (the classic Dieffenbach family covers five generations) indicate that occurrence of the defect is familial and influences males only. This conclusion as to the true sex of the afflicted siblings is strengthened by the fact that among the accumulated offspring, genetic females only are capable of reproducing. Among a total of 137 offspring in 13 families (40), there were 64 normal females, 58 hermaphrodites, and 15 males. The conclusion that the hermaphrodites are genetic males seems to be a reasonable one, and it is gratifying that the new technic of chromatin sex determination has offered confirmation for the conclusion based on the genetic study. It is also reasonable to conclude that all males in these families were affected, since there is but a single male "reported" as having fathered a child.

Recognizing that these individuals are of male genetic sex, it seems likely that an anti-testis factor must interrupt the course of embryogenesis at about mid-term, at a time when differentiation of the duct system is nearing completion and before the external genitalia are organized. In other words, the indifferent gonads become testes, according to genetic constitution, and under their influence the cephalo-caudal progression of Müllerian-duct regression and Wolffian-duct development commences. Before this process is complete, the inductive capacity of the fetal gonads is lost, so that the remaining caudal regions of the secondary sex apparatus, including the external genitalia, are left to differentiate without testicular influence. In this neutral situation, the female condition prevails. It is not certain whether the anti-testis factor acts directly on the gonad, or whether it antagonizes the gonadal secretion which accounts for secondary sex induction. It is certain, however, that such an anti-testis factor does not cause irreparable damage to the gonad itself, for at puberty endocrine function of the gland is awakened, with the production of both estrogen and androgen. The histologic condition of these testes is, as would be expected, that of cryptorchidism. In an exceptional case described by Witschi and Mengert (41), the gonads had descended into scrotal folds and biopsy of the glands demonstrated isolated tubules with spermatogenic activity.

A review of the embryologic situation in male pseudohermaphrodism may be tabulated as follows:

	Male Pseudohermaphrodism	
Embryologic event	*Course of embryogenesis*	*Suggested influence*
Gonadogenesis	Normal (male)	———
Early differentiation of secondary sex characters	Variable differentiation of Wolffian-duct structures	Loss of inductive capacity by fetal testes (maternal anti-testis factor?)
Late differentiation of secondary sex characters	Variable	Loss of inductive capacity by fetal testes (maternal anti-testis factor?)

Gonadal agenesis

The analogy of this situation to *in utero* castration of rabbits has already been discussed. It will be recalled that, in the absence of gonads, the neutral or female condition of the secondary sex structures develops. Several years before the chromatin-mass test for genetic sex became available, Jost suggested that the population of patients with so-called "ovarian" agenesis should reasonably be expected to include both "ovarian" and "testicular" agenesis, for in either case a female habitus would develop (3).

The existence of agonadal genetic males with complete female secondary sex characteristics proves the occurrence of a true, primary agenesis and not a secondary regression of normally differentiated gonads. If the latter had occurred, one would expect to find variable degrees of Wolffian-duct development, depending on the extent of secondary sex differentiation antecedent to regression of the fetal gonads. This is contrary to observations that have been made at laporatomy in these cases.

An analysis of the embryologic events in gonadal agenesis should include some explanation for the observed preponderance of genetic males. Until now, no consideration has been given to a possible role of the germ cells in the aggregate of events leading to sexual abnormalities. On the basis of amphibian experiments, it is justifiable to assume, at least tentatively, that absence or low numbers of primordial germ cells are intermediary causes of abnormal primary sex development (31). This experimental work and its possible application to human gonadal agenesis is discussed in detail in a forthcoming article by Witschi, Nelson and Segal (38). It is concluded that in genetic females, only total absence of germ cells results in gonadal agenesis, whereas in cases of partial depletion of the germ-cell complement, recovery and some degree of ovarian development may occur and thus serve to withdraw a significant number of potential cases from the general classification of gonadal agenesis. Such individuals may later present some degree of infertility, and the group perhaps includes certain women with primary amenorrhea of non-hypophyseal origin, early ovarian failure, or precocious menopause. It is also possible that cases of "true" Klinefelter's syndrome may represent other instances of the "missing" females in the syndrome of gonadal agenesis. In these latter cases, the complement of primordial germ cells may have been reduced not completely, but severely enough to vitiate the degree of recovery that is necessary for any ovarian development. The resultant paucity of germ cells would favor testicular development in spite of female genetic constitution.

Therefore, in genetic females, only a total absence of germ cells would result in gonadal agenesis, whereas in genetic males, both complete deficiency and severely reduced numbers would contribute to the condition. On this basis, one would expect a greater frequency of male genetic sex in

the total number of clinically recognized cases—a fact that has now been established.

Since the possibilities of experimental analysis of sex determination in man are very limited, it must be appreciated that an analysis of the critical events leading to gonadal agenesis can be approached only theoretically. Nevertheless, a reasonable outline of the probable circumstances of sex embryogenesis leading to agonadism can be presented on the basis of studies in experimental embryology:

Gonadal Agenesis

Embryologic event	*Course of embryogenesis*	*Suggested influence*
Gonadogenesis	Does not occur	♂, deficiency or failure of primordial germ cells ♀, failure of primordial germ cells
Early differentiation of secondary sex characters	Female type (in genetic males or females)	Agonadism
Late differentiation of secondary sex characters	Female type (in genetic males or females)	Agonadism

"True" Klinefelter's syndrome

It can no longer be assumed that this type of infertility has its origin in the failure of the seminiferous tubules at puberty to respond to gonadotropic hormone. It would now seem virtually certain that the syndrome has a more primitive origin and becomes manifest with the onset of gonadogenesis. As the developing embryo approaches the critical period of primary sex differentiation, the cortical rudiment in the embryonic gonad undergoes apparent suppression, in spite of female genetic constitution. This suppression must occur without an initial period of rudimentary ovarian differentiation, because even the uppermost portions of the secondary sex apparatus differentiate in the male direction. Thus, in gonads genetically determined to be ovaries, tubular elements develop, and Leydig cells are differentiated under the stimulus of chorionic gonadotropin. The differentiated testis-like structure, in its capacity of embryonic inductor, inhibits completely the development of Müllerian-duct structures. At the same time, Wolffian-duct elements are stimulated to form epididymides, vasa deferentia, and seminal vesicles; the urethra to form the prostate gland; and the primitive external genitalia to form a penis and scrotum. As a consequence, the individual, determined genetically to be a female, is transformed into an apparent male. The underlying problem is why the genetic dominance of the potential embryonic gonadal cortex is not realized. Considering this query in terms of the concept of cortex-medulla antagonism in gonadogenesis,

there are two possible explanations: 1) the genetically epistatic component (in this case, the cortex) experiences a primary failure, thereby allowing the normally repressed component (the medulla) to dominate; and 2) the influence of the hypostatic component (again, the medulla) is reinforced by an external influence, with a resultant successful antagonism by these combined factors over the normally dominant cortex. Although it is not possible to choose between these alternatives at the moment, additional clues will be provided as pre-puberal and fetal cases of Klinefelter's syndrome are discovered. The possibility that a reduced number of primordial germ cells is a significant factor has already been suggested. In testicular biopsy specimens from the youngest series of patients with "true" Klinefelter's syndrome (8 boys ranging in age from 13 to 15 years), rare germinal elements were discernible in only 2 (32). It is recognized that biopsy material is not necessarily representative of the entire gonad, but these observations do indicate the infrequency of germ cells in even the youngest subjects thus far available for study. In the developmental history of the Klinefelter gonad, there appears to be an early inclusion of some genetic female primordial germ cells in the organization of the immature sex gland, but subsequently these cells fail to survive and consequently the gonads may contain few or no germ cells. On the other hand, the germinal cells do not always regress completely, and among those remaining, some may undergo the complete spermatogenic cycle.

Although a definite description of the germ-cell history in the "true" Klinefelter's syndrome must await observations on pre-puberal cases, we can at this time summarize the general developmental events:

	True Klinefelter's Syndrome	
Embryologic event	*Course of embryogenesis*	*Suggested influence*
Gonadogenesis	Dominance of embryonic gonadal medulla (in genetic females)	Primary failure of embryonic cortex (reduced number of germ cells?) or Reinforcement of the medulla by a factor external to the system of medullocortical antagonism
Early differentiation of secondary sex characters	Male type (in genetic females)	Fetal testes
Late differentiation of secondary sex characters	Male type (in genetic females)	Fetal testes

SUMMARY AND CONCLUSIONS

In the final analysis, this discussion of genetic sex and intersexuality may raise more questions than the answers it provides. The complex interrelations of these matters, however, are such that they warrant a definitive statement of the problems surrounding them; an attempt has been made to do this. It is not intended to minimize the importance of various theories at this level of understanding, but it is essential that they be constructed within the entire framework of available observations. Proposals which fit some facts, but ignore others cannot be regarded seriously. It is for this reason that items such as the geneticists' concept of heterochromasy, the principles derived from experimental sex embryology, the endocrine control of secondary sex differentiation, and clinical observations on sex distribution in gonadal agenesis have been given their due and considered place in this attempt to offer at least provisional explanations for some of the more common deviations from normal sexual development.

To a limited extent these explanations may prove helpful to the clinician faced with the problem of establishing the proper sex of rearing for the newborn with ambisexual characteristics. They are in no way intended to imply that genetic sex need be the primary consideration in deciding social sex. Nevertheless, results of a chromatin-mass test can be valuable for early diagnosis of aberrant sexual development and for the establishment of an accurate prognosis. The most frequently encountered problem of this nature arises with the birth of a virilized female infant with an enlarged clitoris and a scrotal-like enlargement of the labial folds. In these instances, the finding of a high urinary excretion of 17-ketosteroids, controllable by the administration of cortisone, is an adequate criterion for accurate diagnosis. Establishment of female genetic sex type, however, obviates the necessity for confirmatory laparotomy. In all other cases of ambisexuality, the ultimate decision as to sex of rearing should depend on the particular physical situation in each case, but knowledge of the genetic sex should be available to the responsible clinician. Armed with this information, he is in a better position to analyze the defect accurately and to predict the sexual development so that his decision as to social sex and possible corrective surgery can be made with confidence.

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The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

A joint session will be held with The American Goiter Association on Thursday morning, May 30, and a joint session with The American Diabetes Association on Saturday morning, June 1. Both of these joint sessions will be held in the meeting room of The Endocrine Society in the Hotel New Yorker. Admittance of members of the A. G. A. to the Thursday morning session, and of members of the A. D. A. to the Saturday morning session will be by badges of their respective societies.

The Committee on Local Arrangements is Dr. Rulon W. Rawson as Chairman with Drs. Earl T. Engle, Joseph W. Jailer, Warren O. Nelson, and Martin Sonenberg as members of the Committee.

All Scientific Sessions will be held in the Hotel New Yorker. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 31, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the New Yorker, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

(For Program, see April issue)

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.

5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE NINTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE MEDICAL COLLEGE OF GEORGIA

AUGUSTA, GEORGIA
October 21–25, 1957

The faculty will consist of 22 eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the Assembly.

For further information concerning the program and registration, write to *Dr. Robert B. Greenblatt, Department of Endocrinology, Medical College of Georgia, Augusta, Georgia.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Bon Air Hotel. Residents and fellows will be admitted for $35.00.

The American Goiter Association

THE 1957 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Statler Hotel, New York City, on *May 28, 29 and 30, 1957*.

PRELIMINARY PROGRAM

HOTEL STATLER

Tuesday, May 28—8:30 a.m.

1. A Study of the Possible Carcinogenic Effect of Internal and External Irradiation of the Thyroid Gland of the Rat.
   V. K. Frantz, E. H. Quimby, M. M. Kligerman and *M. E. Philips*, New York, N. Y.
2. Radiation as a Cause of Thyroid Cancer—Pro and Con.
   B. J. Duffy, Jr., Washington, D. C.
3. Thyroid Carcinomas Persistent After Ten Years.
   W. A. Meissner and *M. A. Legg*, Boston, Mass.
4. Premature Abandonment of Surgical and Radioactive Iodine Therapy in Favor of X-ray Therapy: A Common Error in Treatment of Adenocarcinoma of the Thyroid.
   E. A. Carr, W. H. Beierwaltes and *W. S. Dingledine*, Ann Arbor, Mich.
5. The Use of L-Triiodothyronine as a Pituitary Depressant in the Management of Thyroid Cancer.
   C. G. Thomas, Jr., Chapel Hill, N. Carolina.
6. Results of the Use of Triiodothyronine for the Reduction of Benign Nodular Goiter and the Detection of Thyroid Malignancy.
   W. E. Goodwin and *P. Starr*, Los Angeles, Calif.
7. Scintiscanning for Detection of Thyroid Cancer.
   W. S. Dingledine, E. A. Carr, C. W. Gurney, G. Kleinsasser and *W. H. Beierwaltes*, Ann Arbor, Mich.
8. Pulmonary Fibrosis and Pneumonitis as a Complication of Treatment with I^{131}.
   J. Rall, J. B. Alpers, C. G. Lewallen, M. Sonenberg and *M. Berman*, Bethesda, Maryland, Brookhaven, N. Y., and New York, N. Y.

Tuesday, May 28—2:00 p.m.

9. The Use of I^{133} in the Treatment of Graves' Disease. *M. Berman, D. V. Becker* and *R. S. Benua*, New York, N. Y.
10. Clinical Progress in the Handling of Malignant Exophthalmos.
    E. P. McCullagh, Cleveland, Ohio.
11. T.S.H. Discharge of Radioiodine from Thyroid Nodules in vitro.
    Selwyn Taylor and *B. Moffitt*, London, England.
12. Studies in Non-Myxedematous Hypometabolism. II. The Turnover of I-131 Labelled Thyroxine After Intravenous Infusion.
    G. S. Kurland, J. G. Bustos, M. Hamolsky and *A. S. Freedberg*, Boston, Mass.
13. The Relative Binding Capacity of Human Plasma Human Serum Albumin, and Human Erythrocytes for Radioactive L-Thyroxine and L-Triiodothyronine.
    K. R. Crispell, H. Hyer and *J. Coleman*, Charlottesville, Va.

14. Comparison of Clinical Value of Serum Protein Bound or Butyl Extractable Iodines.
E. B. Man and *P. K. Bondy*, New Haven, Conn.
15. Further Observations of the Effects of Iodides on the Thyroid Hormone Secretion Rate in Thyrotoxic Subjects.
R. E. Goldsmith, G. Lutsch and *C. Herbert*, Cincinnati, Ohio.
16. An Effect of Thyroid Function on Androgen Metabolism.
L. Hellman, B. Zumoff, L. Bradlow and *T. F. Gallagher*, New York, N. Y.

Wednesday, May 29—8:30 a.m.

17. Thyrotrophin Potency of Transplantable Pituitary Tumors of Mice Through Four Transfers.
R. W. Bates, P. G. Condliffe, E. Anderson and *J. Furth*, Bethesda, Md.
18. Effect of Various Levels of Thyrotrophin Secretion on Thyroid Size, Structure and Radioiodide Concentration (T/S).
E. M. Bogdanove and *J. N. Bogdanove*, Albany, N. Y.
19. The Metabolism of Thyroidal Phospholipids with Special Reference to the Mechanism of Action of Pituitary Thyrotropin.
N. Freinkel, Boston, Mass.
20. The Metabolism of 3-3'-Diiodothyronine in Man.
J. B. Stanbury and *M. L. Morris*, Boston, Mass.
21. In Vitro Action of Thyroxine Analogs on Succinate and Malate Oxidation.
S. B. Barker, Birmingham, Ala.
22. The Autoradiographic Localization of Thyroid Hormones in Organs and Tissues.
D. H. Ford and *J. Gross*. Brooklyn, N. Y.
23. The Uptake and Metabolism of S^{35} Labeled Thiourea and Thiouracil by the Thyroid and Other Tissues.
F. Maloof and *M. Soodak*. Boston, Mass.
24. Thyroxine Excretion, A Possible Cause of Goiter.
L. Van Middlesworth, Memphis, Tenn.

Wednesday, May 29—2:00 p.m.

25. Presidential Address: B. M. Dobyns, Cleveland, Ohio.
26. Van Meter Prize Essay.
27. Serum Protein Abnormalities in Thyroid Disease.
D. C. Morgan, R. Bird and *R. Greene*, Hampstead, England.
28. Granulomatous Thyroiditis: A Clinico-Pathologic Study of Histologically Proven Cases.
L. B. Woolner, W. M. McConahey and *O. H. Beahrs*, Rochester, Minn.
29. Pathologic Studies in Adult Myxedema.
R. C. Douglass and *S. D. Jacobson*, Highland Park, Mich.
30. Adrenocortical Function in Myxedema.
G. A. William, K. R. Crispell and *W. Parson*, Charlottesville, Va.

Thursday, May 30—9:30 a.m.

JOINT SESSION WITH THE ENDOCRINE SOCIETY

Grand Ballroom
Hotel New Yorker

31. A Quantitative Comparison of the Response to Exogenous Thyrotropin in Euthyroid and Thyrotoxic Human Subjects.

M. A. Greer and *F. H. Shull*, Bethesda, Md.

32. Distribution of Thyroidal Iodine in Some Nodular Goiters.
R. Pitt-Rivers, D. Hubble and *W. H. Hoather*, London, England.
33. The Effect of Thyrotropin on the Serum Pattern of Thyroid Hormones.
J. D. Hydovitz and *W. L. Arons*. Pittsburgh, Pa.
34. Metabolites of L-Triiodothyronine and L-Thyroxine.
E. V. Flock, J. L. Bollman, J. H. Grindlay and *B. F. McKenzie*, Rochester, Minn.
35. Effect of Triiodothyronine and Triiodothyroacetic Acid on Cortisol Production and Metabolism in the Dog.
J. C. Melby, R. H. Egdahl and *W. W. Spink*, Minneapolis, Minn.
36. Interactions of Hydrocortisone and Thyrotropin on the Hormonal Secretion from the Thyroid of Intact Dogs.
N. B. Ackerman, J. M. Miller and *R. W. Smith, Jr.*, Detroit, Mich.
37. Clinical Studies of an Inhibitor of TSH in Human Urine.
P. Starr and *A. Lepp*, Los Angeles, Calif.
38. Placental Transfer of Thyroxine and Triiodothyronine.
E. Knobil and *J. B. Josimovich*, Boston, Mass.
39. Studies of Iodinated Compounds in the Blood in Cases of Nontoxic Goiter.
J. J. Rupp, C. Chavarria, J. Brunet and *K. E. Paschkis*, Philadelphia, Pa.

GRANTS OF RACEMIC ALDOSTERONE

U. S. Department of
Health, Education, and Welfare
Public Health Service,
National Institutes of Health,
Bethesda 14, Maryland

Ciba Pharmaceutical Products, Inc., Summit, New Jersey, has made available to the Endocrinology Study Section, National Institutes of Health (Bethesda 14, Maryland), its limited supply of *d,l*-aldosterone for biological and clinical investigation. This synthetic racemic material has half the biological activity of the natural *d*-aldosterone.

It is available to qualified investigators in the following forms:

d,l-aldosterone 21-monoacetate
(purified sesame oil solution)—200 micrograms/ml.
(purified sesame oil solution)— 20 micrograms/ml.
d,l-aldosterone 21-monoacetate
(95 per cent ethanol solution)— 1 milligram/ml.
d,l-aldosterone (non-esterified)
(95 per cent ethanol solution)—10 micrograms/ml.

The monoacetate will be distributed for biological work and the nonesterified form limited to use as a chromatographic standard. The alcohol solution of the monoacetate can be diluted for intravenous injections. Purified sesame oil can be obtained from CIBA for those who have occasion to dilute the oil solutions.

Requests should be made to Dr. R. T. Hill, Executive Secretary, Endocrinology Study Section, by letter briefly stating the exact amount wanted and the nature of the investigation.

CORRECTION

The author wishes to make two corrections in Table 1 of the following article—Eik-Nes, Kristen: Determination of 17,21-Dihydroxy-20-ketosteroids in Blood Plasma, *J. Clin. Endocrinol. & Metab.* **17**: 502–511 (April) 1957. In the last column of Table 1, the value for aliquot No. 4 should be .087 instead of .887; and the first footnote to the table should read "Δ = Twice the reading at 410 mμ *less* the sum of the readings at 370 mμ and 450 mμ."

BOOK REGISTER

Clinical Physiology. The Functional Pathology of Disease. Edited by ARTHUR GROLLMAN, M.D., PH.D., Professor and Chairman of the Department of Experimental Medicine, University of Texas Southwestern Medical School, Dallas, Texas. Twenty-five other contributors are all engaged in the practice of medicine and have made outstanding contributions to advancement in their respective fields of medicine. In this book, the basic physiologic principles are correlated with clinical medicine, in diagnosis and treatment. Dr. Grollman states "The physicians of today cannot apply the knowledge available in clinical medicine without a thorough understanding of the physiologic principles upon which this knowledge is based." Emphasis is given to metabolic, cardiovascular, and endocrine discussions, since these are the areas in which the greatest advances have been made in recent years; 870 pages; 32 tables; 30 figures; 1957. McGraw-Hill Book Company, Inc., New York, N. Y. Price $12.50.

Congenital Anomalies of the Viscera: Their Embryological Basis. By J. LEWIS BREMER, M.D., Hersey Professor of Anatomy, Emeritus, Harvard University, and The Children's Hospital, Boston, Massachusetts. This authoritative work presents a large variety of clinically important prenatal and neonatal anomalies from the embryological point of view, and is the first adequate account of this subject as a whole. The delicate adjustments needed for normal development are stressed, for without these adjustments anomalies are certain to occur. Potential causes of changes in the growth rate of the embryo are discussed. New types of abnormalities and unusual types hitherto unexplained can now frequently be related to their embryological sources; 212 pages; 89 figures; 1957. Harvard University Press, Cambridge, Massachusetts. Price $5.00.

Expectant Motherhood. Third edition, revised. By NICHOLSON J. EASTMAN, M.D., Professor of Obstetrics in Johns Hopkins University; and Obstetrician-in-Chief to The Johns Hopkins Hospital, Baltimore, Maryland. This new edition of an authoritative guidebook through pregnancy includes up-to-the-minute information on diet, prevention of overweight, and new developments in anesthesia for childbirth. It maintains a clear and well balanced approach to pregnancy, and is readily understood by the average patient; 210 pages; 13 figures; 1957. Little, Brown and Company, Boston, Massachusetts. Price $1.75.

Liver, Biliary Tract and Pancreas. Part III of Volume 3, Digestive System. The Ciba Collection of Medical Illustrations. By FRANK H. NETTER, M.D. The whole collection of these authentic, full-color illustrations is estimated to be a 9-volume twenty-year project, edited by DR. ERNEST OPPENHEIMER. Volume 3 is being developed in three parts, and it was decided to publish this third part, before the other two, because the subject matter is of such interest to the medical profession. The topics include the normal anatomy of the liver, biliary tract and pancreas, signs and symptoms of disease, diagnostic tests, congenital anomalies, host-parasite relationships, and blood supply to tissues. Part III incorporates a new feature, namely, literature references. The 165 pages include 133 full-color plates by Dr. Netter. In the United States, copies may be obtained from the Publications Department, Ciba Pharmaceutical Products Inc., Summit, N. J., and also at certain bookstores which are permitted to stock the volume. Sold at cost, $10.50.

Practical Dermatology. By SAMUEL M. PECK, B.S., M.D., Dermatologist to the Mount Sinai Hospital, New York, Associate Clinical Professor of Dermatology, Columbia University, New York, N. Y., with LAURENCE L. PALITZ, M.D., PH.D., Attending Dermatologist, Long Island Jewish Hospital, Assistant Attending Dermatologist, Mount Sinai Hospital, New York, N. Y. This is the sixth volume in the Series of "Handbooks for the General Practitioner." As the authors state in their preface, it is "a survey of dermatologic disorders as they are commonly met with in daily practice." Only those measures with which the authors "have had thorough and long enough experience" are recommended. Discussions are included on cancerous conditions of the skin, syphilis, acne, vitamin deficiencies, dermatophytosis, and the skin manifestations of endocrine disease; 397 pages; 122 illustrations; 1956. Distributed solely by McGraw-Hill Book Co., New York, N. Y., for Landsberger Medical Books, Inc. Price $7.00.

The Importance of Overweight. By HILDE BRUCH, M.D., Associate Clinical Professor of Psychiatry, College of Physicians and Surgeons, Columbia University, New York, N. Y. A comprehensive discussion of the medical, biological, sociological and emotional factors involved in obesity; 448 pages; 1957. W. W. Norton and Company, Inc., New York, N. Y. Price $5.95.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Cold Injury. Fourth Conference, November 7–9, 1955, Princeton, N. J. Edited by M. IRENÉ FERRER, M.D., Assistant Professor of Clinical Medicine, Columbia University College of Physicians and Surgeons, New York, N. Y. Includes discussions of such topics as cold injury cases from the Korean War, roentgenologic changes in bones following cold injury in man, experimental frostbite in rabbits, follow-up report on cases of cold injury from World War II, resuscitation of hypothermic and frozen mammals, clinical application of hypothermia, and cerebral studies during hypothermia; 371 pages; 134 illustrations, some in color; 20 tables; 1956. Price $5.95.

Gestation. Third Conference, March 6–8, 1956, Princeton, N. J. Edited by CLAUDE A. VILLEE, Assistant Professor of Biological Chemistry, Harvard Medical School, Boston, Massachusetts. Several authors who are prominent in their field of research discuss the following—Maternal endocrine secretions, maternal hormones in pregnancy, ovulation cycles and stress, the secretion of various hormones and the activity of the adrenal cortex in pregnancy, steroidogenesis in perfused human placentas, effect of hormones on cellular metabolism, etiology of gonadal agenesis and sex reversal, the secretory activities of fetal endocrine glands and their effect upon target organs, influence of hormones on sex differentiation in explanted fetal reproductive tracts, effect of fetal endocrines on fetal growth, and interrelations of pituitary functions and pregnancy; 253 pages; 89 figures; 25 tables; 1957. Price $4.75.